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04.02.2004

REC'D 23 APR 2004

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Patentanmeldung Nr. Patent application No. Demande de brevet n°

03075331.3

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R C van Dijk



Anmeldung Nr:
Application no.: 03075331.3
Demande no:

Anmeldetag:
Date of filing: 04.02.03
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se référer à la description.)

Plant promoters

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s)
revendiquée(s)

Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

EP/23.12.02/EP 02080651

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

C12N15/00

Am Anmeldetag benannte Vertragsstaaten/Contracting states designated at date of
filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL
PT SE SI SK TR LI

PLANT PROMOTERS

The present invention relates to the field of plant molecular biology, more particularly to promoter sequences useful for gene expression in plants. The isolation of these promoter sequences from rice and barley, as well as their use for regulating the expression of a gene is disclosed.

Initiation of transcription is generally understood to be the predominant controlling factor in determining expression of a gene. The transcriptional control elements, which may interact with DNA binding proteins, are generally embedded in the sequence 5'-flanking or upstream of the transcribed gene. These DNA sequence elements promote the formation of transcriptional regulatory complexes that either activate or repress the expression of the 3' downstream gene. Therefore these regulatory sequences are called "promoters".

Within the sequence of the promoter, many regulatory sequence elements can be embedded that control the promoter activity. Some of them are called control elements or "boxes" and are recognition sites to which regulatory transcription factors can bind.

Transformation of a DNA sequence into a host cell and expression of a transgene in a host cell, tissue or organism is common practice in recombinant DNA technology. The choice of promoter, operably linked to the transgene, will determine when, where or how strong the transgene is expressed in the host cell or host organism. Frequently those skilled in the art desire to limit the expression of the transformed DNA sequence to a certain time-period, or during certain environmental circumstances, or to a defined tissue or organ of the host, or to a certain expression level. Especially in the field of plant molecular biology, controlled expression of genes in transgenic plants may have several advantages over ubiquitous and constitutive expression and may contribute to obtaining a useful phenotype of the transgenic plant. There is a broad need for diverse transcriptional control elements capable of regulating specific expression of genes in plants. It is therefore important that such regulatory elements are isolated and that their specific transcriptional features are characterized.

Known procedures to identify new regulatory elements are based on in silico studies of the genomic sequences that are provided in public databases. However, the determination of the actual location of these elements, using only computer-assisted methods, is highly error prone, even for the best-characterized promoter control elements such as TATA box and transcription

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initiation element (INR). Also the determination of the exact prediction of the coding sequence and the correct start codon, using only computer-assisted methods, is error-prone. Visual investigation of the sequence, by the skilled person, can help for defining the position of the coding sequence, but the prediction of the specificity and regulation of the promoter is to date extremely speculative and almost bound to fail.

In silico and in vitro investigation of a promoter, to determine its specificity and/or regulation, can be performed for example by the following steps (i) investigation of the expression of the corresponding mRNA, by Northern blotting, RT-PCR or micro-array etc.; (ii) investigation of the expression of the corresponding protein, by Western blotting or protein analysis etc. (iii) data mining of EST libraries (in silico Northern blot) (iv) expression of a reporter gene under the control of the isolated promoter of interest, in a transgenic organism. The last step is susceptible to give unambiguous data about the expression pattern of a promoter, on different levels such as the type of organism, cell type or tissues type, during the complete life cycle of the organism or under which different conditions.

In biotechnology, the expression of a gene of interest into a transgenic organism can give different results depending of the promoter used. Thus, the choice of an adequate promoter can be critical for obtaining the desired effect in the transgenic organism. Therefore, the identification, isolation and precise characterization of promoters are of great importance in genetic engineering.

In the present invention it is now described how new promoters were isolated and characterized. More particularly, the isolation and characterization of a collection of promoters originating from rice (*Oryza sativa*) and from Barley (*Hordeum vulgare*), with various expression patterns, are described. In this invention, the promoters are isolated as DNA regions spanning about 1.2 kb of the sequence upstream of the translation initiation codon (i.e. first ATG), this codon excluded, from various rice or barley genes.

Many of these isolated nucleic acids were not isolated before and/or their sequence, i.e. the functional region that comprised the promoter activity, was not revealed before. Further, for many of these isolated nucleic acids, their functionality and use as a promoter has not been described and/or their precise expression pattern when used as a promoter to drive an operably linked nucleic acid was not described before.

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This isolated nucleic acids of approximately 1.2kb are isolated from the upstream region of the genes and include therefore the 5'UTR of the pre-messenger RNA, the transcription initiation element (INR) and the core or minimal promoter and likely contain most of the critical regulatory elements situated more upstream.

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Therefore the sequences as well as a fragment thereof are subject of the present invention and these fragments are useful in practicing the methods of the present invention.

10 Accordingly; in a first embodiment, the present invention provides an isolated nucleic acid, capable of regulating transcription of an operably linked nucleic acid, comprising a sequence or a fragment thereof, said sequence being selected from the group consisting of:

- (a) a nucleic acid comprising the DNA sequence as given in any of SEQ ID NOs 1 to 69 or the complement thereof,
- (b) a nucleic acid specifically hybridizing with the nucleotide sequence as defined in (a),
- 15 (c) a nucleic acid which is diverging, due to the differences between alleles, to a nucleotide sequence as defined in (a) or (b),
- (d) a nucleic acid as defined in any one of (a) to (c), said nucleic acid interrupted by intervening DNA,
- (e) a nucleic acid, which is obtainable from plant DNA, said plant DNA comprising a plant gene,
- 20 which plant gene hybridizes specifically with a sequence as represented by any one of SEQ ID NO 208 to 276 ;
- (f) a nucleic acid, which is obtainable from plant DNA, said plant DNA comprising a plant gene, which plant gene is homologues to a sequence as represented by any one of SEQ ID NO 208 to 276, preferably said plant gene is 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%
- 25 homologous;
- (g) a nucleic acid, which is obtainable from plant DNA, said plant DNA comprising a plant gene, which plant gene is encoding a protein which is homologous to a protein encoded by any one of the sequences represented by SEQ ID NO 208 to 276, preferably said plant protein is 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% homologous;
- 30 provided that said isolated nucleic acid is not one of the nucleic acids as deposited in the Genbank database under any of the Genbank Accession numbers which are listed in Table 1.

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Regulating transcription comprises increasing or decreasing the level of transcription of a nucleic acid, such as a gene. In a particular embodiment of the invention regulation of expression results in increased transcription of the operably linked nucleic acid. This may result in ectopic expression

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and/or overexpression of an operably linked nucleic acid, whereby the level of expression of the operably linked nucleic acid is increased compared to the natural level of expression. This increase of expression level compared to the natural expression level is not necessary throughout the whole organism and at any time, but it can be limited to certain cell types, tissues or organs, or during a certain period of time or in response to certain environmental conditions. Furthermore the results of this regulation of transcription, for example ectopic expression and/or overexpression of a nucleic acid, can be the increased activity of the protein encoded by the operably linked nucleic acid, or alternatively, it can be the reduced activity of the protein encoded by the operably linked nucleic acid. The latter situation will occur when for example the operably linked nucleic acid initiates cosuppression or when the operably linked nucleic acid is an antisense nucleic acid.

The term "operably linked" to a nucleic acid of the present invention means connected in such a way that it can be influenced by the function of the nucleic acid of the present invention. More particularly, "operably linking" according to the present invention means, being controlled by the promoter of the present invention. For the purposes of genetic engineering of a host cell, the promoter of the present invention is introduced into the host cell and it is operably linked to a nucleic acid, which can be an introduced transgene or which can be an endogenous gene from the host.

A fragment as used herein means any part, region or portion of the nucleic acid sequence. Preferably, these fragments contain a certain functional element of the nucleic acid according to the present invention. Such fragments can be identified by standard techniques well known by the skilled person e.g. by the following method. The nucleic acid of the present invention, can be randomly mutated to block the function of certain regulatory element in the nucleic acid. Alternatively, deletion mutants can be made, whereby the nucleic acid is shortened by for example 10 bp or any multiple of 10bp, starting from the 5' end of the nucleic acid. Subsequently the mutated promoters are operably linked to a reporter gene and the expression pattern is analysed in a host cell. If the mutated version shows a changed expression pattern, then the mutated or deleted portion was responsible for the changed expression pattern. In a particular embodiment of the present invention, such a fragment is a regulatory element or a box, or such fragment is the minimal promoter. With minimal promoter is meant, a smaller fragment of the promoter, which is still capable of promoting the initiation of transcription of the operably linked DNA. In a particular embodiment of the present invention, the fragments as described above are used to make hybrid or chimeric promoters.

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A nucleic acid sequence as used and described in the present invention is an (isolated) DNA, cDNA, genomic DNA, synthetic DNA, or RNA wherein T is replaced by U.

Differences between alleles are naturally occurring differences between the genes of different plants of the same species. These differences can be substitution and/or addition and/or deletion of 1, 2, 3 or more base pairs.

Nucleic acid molecules as defined in the current invention can be interrupted by intervening sequences. With "intervening sequences" is meant any nucleic acid sequence which disrupts the sequence of the present invention or which disrupts the functional format of a nucleic acid molecule according to the present invention, for example disrupting the operably linkage of the operably linked nucleic acid. Removal of the intervening sequence may restore the sequence or the functional format thereof. Examples of intervening sequences comprise Introns, mobilizable nucleic acids sequences such as transposons or nucleic acid tags such as e.g. a T-DNA or nucleic acids that can be mobilized as the result of a recombination event.

The present invention also encompasses promoters of homologous plant gene. These promoters can be isolated from a plant DNA that comprises a sequence which sequence hybridises with the nucleic acid sequence which is naturally under the control of a promoter of the present invention, meaning the sequences as presented by any of SEQ ID NO 208 to 276 or fragments thereof. These promoters of homologous genes of the same plant species or from different plant species are also useful in practicing the methods according to the invention. The term "hybridisation" as defined herein is a process wherein substantially homologous complementary nucleotide sequences anneal to each other. Tools in molecular biology relying on such a process include the polymerase chain reaction (PCR; and all methods based thereon), subtractive hybridisation, random primer extension, nuclease S1 mapping, primer extension, reverse transcription, cDNA synthesis, differential display of RNAs, and DNA sequence determination, Northern blotting (RNA blotting), Southern blotting (DNA blotting). The hybridisation process can also occur with one of the complementary nucleic acids immobilised to a matrix such as magnetic beads, Sepharose beads or any other resin. Tools in molecular biology relying on such a process include the isolation of poly (A+) mRNA. The hybridisation process can furthermore occur with one of the complementary nucleic acids immobilised to a solid support such as a nitro-cellulose or nylon membrane or immobilised by e.g. photolithography to, for example, a siliceous glass support (the latter known as nucleic acid arrays or microarrays or as nucleic acid chips). Tools in molecular biology relying on such a process include RNA and DNA gel blot analysis, colony hybridisation,

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plaque hybridisation, *in situ* hybridisation and microarray hybridisation. In order to allow hybridisation to occur, the nucleic acid molecules are generally thermally or chemically denatured to melt a double strand into two single strands and/or to remove hairpins or other secondary structures from single stranded nucleic acids. The stringency of hybridisation is influenced by conditions such as temperature, salt concentration and hybridisation buffer composition. High stringency conditions for hybridisation include high temperature and/or low sodium/salt concentration (salts include the sodium as for example in NaCl and Na₃-citrate) and/or the inclusion of formamide in the hybridisation buffer and/or lowering the concentration of compounds such as SDS (sodium dodecyl sulphate detergent) in the hybridisation buffer and/or exclusion of compounds such as dextran sulphate or polyethylene glycol (promoting molecular crowding) from the hybridisation buffer. Conventional hybridisation conditions are described in, for example, Sambrook (2001) Molecular Cloning: a laboratory manual, 3rd Edition Cold Spring Harbor Laboratory Press, CSH, New York, but the skilled craftsman will appreciate that numerous different hybridisation conditions can be designed in function of the known or the expected homology and/or length of the nucleic acid sequence. Specifically hybridising refers to hybridising under stringent conditions, i.e. at a temperature of 60°C followed by washes in 2XSSC, 0.1XSDS, and 1X SSC, 0.1X SDS. Sufficiently low stringency hybridisation conditions are particularly preferred for the isolation of nucleic acids heterologous to the DNA sequences of the invention defined supra. Elements contributing to heterology include allelism, degeneration of the genetic code and differences in preferred codon usage.

The invention also relates to a nucleic acid molecule of at least 15 nucleotides in length hybridizing specifically with any of the nucleic acids of the invention. The invention also relates to a nucleic acid molecule of at least 15 nucleotides in length specifically amplifying a nucleic acid of the invention by polymerase chain reaction.

According to another embodiment of the present invention there is also provided the promoters of homologous plant gene. These promoters can be isolated from a plant DNA that comprises a sequence which is homologous to the nucleic acid sequence which is naturally under the control of a promoter of the present invention, meaning homologous the sequences as presented by any of SEQ ID NO 208 to 276 or fragments thereof. These promoters of homologous genes, originating from the same or different plant species, are useful in practicing the methods according to the invention. Methods for the search and identification of such homologous genes, would be well within the realm of a person skilled in the art. Such methods involve alignment and comparison sequences using softwares or algorithms such as GAP, BESTFIT, BLAST, FASTA and TFASTA.

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GAP uses the algorithm of Needleman and Wunsch (J. Mol. Biol. 48: 443-453, 1970) to find the alignment of two complete sequences that maximizes the number of matches and minimizes the number of gaps. The BLAST algorithm calculates percent sequence identity and performs a statistical analysis of the similarity between the two sequences. The software for performing BLAST analysis is publicly available through the National Center for Biotechnology Information.

In a particular embodiment of the present invention, the homologous genes as described above belong to the same gene family as the gene corresponding to the any of the sequences SEQ ID NO 208 to 276. The analysis of a gene family can be carried out by sequence similarity analysis. To perform this analysis one can use standard programs for multiple alignments e.g. Clustal W. This analysis can be done on the full-length sequence or based on a comparison of certain regions such as conserved domains.

Within the same gene family, the promoters may have sufficient sequence homology so to search for homologous promoters immediately with the promoter sequence according to the present invention, i.e. SEQ ID NO 1 to 69, instead of with the sequence of the gene naturally controlled by these promoters, i.e. SEQ ID NO 208 to 276. This method is particularly useful to isolated promoters of the same plant species, but a different variety.

The genome sequences of *Arabidopsis* and rice are now available in public databases such as Genbank and other genomes are currently being sequenced. Therefore, it is expected that as more sequences of genomes of other plants will become available, many other promoters of homologous genes will be identifiable by sequences alignment with any one of SEQ ID NO 208 to SEQ ID NO 276. Therefore the skilled person will be in the possibility to find the promoters of the homologous genes from other plant species. The promoters of homologous genes of crop plants are especially useful for practicing the methods of the present invention in crop plants.

Alternatively or additionally, homologous genes may be found, based on the sequence comparison of the proteins of fragments thereof, which they encode, with the protein sequence encoded by the nucleic acid sequences represented by any of SEQ ID NO 208 to 276. "Homologues" of protein encoded or partially encoded by any of the sequences SEQ ID NO 208 to 276 or fragments thereof, encompass peptides, oligopeptides, polypeptides, proteins and enzymes having amino acid substitutions, deletions and/or insertions relative to that encoding protein corresponding to SEQ ID NO 208 to 276 and having similar biological and functional activity as the unmodified protein from which they are derived. This method for finding promoters, allows

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identification and isolation of promoters of the same gene in relatively distant organism, meaning organisms having large genetic divergence due to evolution. Therefore this method allows the identification of promoters of orthologous and paralogous to nucleic acids as represented by any of SEQ ID NO 208 to 276.

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Two special forms of homology, orthologous and paralogous, are evolutionary concepts used to describe ancestral relationships of genes. The term "paralogous" relates to gene-duplications within the genome of a species leading to paralogous genes. The term "orthologous" relates to homologous genes in different organisms due to ancestral relationship. The term "homologues" as used herein also encompasses paralogues and orthologues of the proteins useful in the methods according to the invention.

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The gene or protein homologues useful in the method according to the invention have at least 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 54, 56, 58, % sequence identity or similarity (functional identity) to the gene or protein corresponding to any of SEQ ID NO 208 to 276, alternatively at least 60, 62, 64, 66, 68, % sequence identity or similarity to the gene or protein corresponding to any of SEQ ID NO 208 to 276, or alternatively at least 70, 72, 74, 76, 78% sequence identity or similarity to the gene or protein corresponding to any of SEQ ID NO 208 to 276. Typically, the homologues have at least 80, 82, 84% sequence identity or similarity, preferably at least 85, 86, 88% sequence identity or similarity, further preferably at least 90, 92, 94, 96, 98% sequence identity or similarity to the gene or protein corresponding to any of SEQ ID NO 208 to 276, most preferably at least 95% sequence identity or similarity to the gene or protein corresponding to any of SEQ ID NO 208 to 276.

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These levels homologies can be calculated by using a pairwise alignment such as the program Align X (December 8, 1999) a component of the vector NTI suite 5.5 (Informax, Inc.) using the standard parameters or using the parameters gap opening penalty 15 and gap extension penalty 6.66.

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It is obvious for the person skilled in the art that the promoters according to the present invention, i.e. SEQ ID NO 1 to 69 or the promoters of homologous genes, can be used for the regulation of expression of an operably linked gene as such, in part, or as comprised into a larger DNA fragment. A promoter sequence according to the invention cannot easily be defined in number of basepairs and therefore the present invention extends to nucleic acids comprising a sequence essentially similar to as sequence as presented by any of SEQ ID NO 1 to 69 or a part thereof.

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However, some sequences comprising SEQ ID NO 1 to 69 or a part thereof are already known,

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such as the very large BAC clones available in the public Genbank databases, or such as the cloned genes available in the Genbank database. It is to be understood that these publicly available sequences are not part of the invention, since these large sequences, or the cloned genes cannot be functional for regulation of the expression of a nucleic acid, when said nucleic acid is operably linked to those public (large) sequences.

Further the present invention encompasses an isolated nucleic acid according as mentioned above, capable of regulating transcription of an operably linked nucleic acid in a plant.

The term "plant" as used herein encompasses whole plants, ancestors and progeny of the plants and plant parts, including seeds, shoots, stems, roots (including tubers), and plant cells, tissues and organs. The term "plant" also Therefore encompasses suspension cultures, embryos, meristematic regions, callus tissue, leaves, seeds, roots, shoots, gametophytes, sporophytes, pollen, and microspores. Plants that are particularly useful in the methods of the invention include all plants which belong to the superfamily *Viridiplantae*, in particular monocotyledonous and dicotyledonous plants including a fodder or forage legume, ornamental plant, food crop, tree, or shrub selected from the list comprising *Acacia* spp., *Acer* spp., *Actinidia* spp., *Aesculus* spp., *Agathis australis*, *Albizia amara*, *Alsophila tricolor*, *Andropogon* spp., *Arachis* spp., *Areca catechu*, *Astelia fragrans*, *Astragalus cicer*, *Baikiaea plurijuga*, *Betula* spp., *Brassica* spp., *Brugulera gymnorhiza*, *Burkea africana*, *Butea frondosa*, *Cadaba farinosa*, *Calliandra* spp., *Camellia sinensis*, *Canna indica*, *Capsicum* spp., *Cassia* spp., *Centroema pubescens*, *Chaenomeles* spp., *Cinnamomum cassia*, *Coffea arabica*, *Colophospermum mopane*, *Coronillia varia*, *Cotoneaster serotina*, *Crataegus* spp., *Cucumis* spp., *Cupressus* spp., *Cyathea dealbata*, *Cydonia oblonga*, *Cryptomeria japonica*, *Cymbopogon* spp., *Cynthea dealbata*, *Cydonia oblonga*, *Dalbergia monetaria*, *Davallia divaricata*, *Desmodium* spp., *Dicksonia squarosa*, *Diheteropogon amplexans*, *Dioclea* spp., *Dolichos* spp., *Dorycnium rectum*, *Echinochloa pyramidalis*, *Ehrartia* spp., *Eleusine coracana*, *Eragrestis* spp., *Erythrina* spp., *Eucalyptus* spp., *Euclea schimperi*, *Eulalia villosa*, *Fagopyrum* spp., *Feijoa sellowiana*, *Fragaria* spp., *Flemingia* spp., *Freycinetia banksii*, *Geranium thunbergii*, *Ginkgo biloba*, *Glycine javanica*, *Gliricidia* spp., *Gossypium hirsutum*, *Grevillea* spp., *Guibourtia coleosperma*, *Hedysarum* spp., *Hemarthia altissima*, *Heteropogon contortus*, *Hordeum vulgare*, *Hyparrhenia rufa*, *Hypericum erectum*, *Hyperthelia dissoluta*, *Indigo incarnata*, *Iris* spp., *Leptarrhena pyrolifolia*, *Lespedeza* spp., *Lettuca* spp., *Leucaena leucocephala*, *Loudetia simplex*, *Lotonous bainesii*, *Lotus* spp., *Macrotyloma axillare*, *Malus* spp., *Manihot esculenta*, *Medicago sativa*, *Metasequoia glyptostroboides*, *Musa sapientum*, *Nicotianum* spp., *Onobrychis* spp., *Ornithopus* spp., *Oryza* spp., *Peltophorum africanum*, *Pennisetum* spp., *Persea gratissima*, *Petunia* spp., *Phaseolus* spp., *Phoenix canariensis*, *Phormium cookianum*, *Photinia*

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spp., *Picea glauca*, *Pinus* spp., *Plum sativum*, *Podocarpus totara*, *Pogonarthria flecklii*, *Pogonarthria squarrosa*, *Populus* spp., *Prosopis cineraria*, *Pseudotsuga menziesii*, *Pterolobium stellatum*, *Pyrus communis*, *Quercus* spp., *Raphiolepis umbellata*, *Rhopalostylis sapida*, *Rhus natalensis*, *Ribes grossularia*, *Ribes* spp., *Robinia pseudoacacia*, *Rosa* spp., *Rubus* spp., *Salix* spp., *Schyzachyrium sanguineum*, *Sciadopitys verticillata*, *Sequoia sempervirens*, *Sequoiadendron giganteum*, *Sorghum bicolor*, *Spinacia* spp., *Sporobolus fimbriatus*, *Stiburus alopecuroides*, *Stylosanthos humilis*, *Tadehagi* spp, *Taxodium distichum*, *Themeda triandra*, *Trifolium* spp., *Triticum* spp., *Tsuga heterophylla*, *Vaccinium* spp., *Vicia* spp. *Vitis vinifera*, *Watsonia pyramidata*, *Zantedeschia aethiopica*, *Zea mays*, amaranth, artichoke, asparagus, broccoli, brussel sprout, cabbage, canola, carrot, cauliflower, celery, collard greens, flax, kale, lentil, oilseed rape, okra, onion, potato, rice, soybean, straw, sugarbeet, sugar cane, sunflower, tomato, squash, and tea, trees and algae amongst others. According to a preferred feature of the present invention, the plant is a monocotyledonous plant, further preferably a cereal, most preferably a plant selected from rice, maize, wheat, millet, barley, rye, oat.

Further the invention encompasses the isolated nucleic acid as mentioned above, wherein the isolated nucleic acid is a promoter.

Reference herein to a "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences of eukaryotic genes, with or without the classical TATA box, with or without a CCAAT box sequence and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue-specific manner.

The term "promoter" may also include the transcriptional regulatory sequences of a classical prokaryotic gene, in which case it may include a -35 box sequence and/or a -10 box transcriptional regulatory sequences.

The term "promoter" is also used to describe a synthetic or fusion molecule, or derivative capable of regulating transcription of a nucleic acid molecule in a cell, tissue or organ.

In the context of the present invention, the promoter preferably is a plant-expressible promoter sequence. Promoters, however, that also function or solely function in non-plant cells such as bacteria, yeast cells, insect cells and animal cells are not excluded from the invention. By "plant-expressible" is meant that the promoter sequence, including any additional regulatory elements added thereto or contained therein, is at least capable of regulating expression of an operably linked DNA in a plant cell, tissue or organ.

In the present invention, the particular expression patterns of the promoters according to the present invention are revealed and therefore contribute to the art by providing tools to specifically regulate transcription of genes. Accordingly, the invention encompasses, an isolated nucleic acid as mentioned above, capable of regulating transcription of an operably linked DNA sequence in one or more particular cells, tissues or organs of a plant. In a more specific embodiment of the invention the expression is regulated in the seed, embryo, scutellum, aleurone, endosperm, leaves, flower, calli, meristem, discriminating centre, shoot, shoot meristem and root.

A discriminating centre includes the meristematic zone, from where root and shoot originate.

Distinct classes of promoters can be defined. There are promoters that are active in all parts of the organism, and not much responsive to regulations by endogenous or exogenous signals, such as aging, stresses, nutritional status etc. Alternatively, there are regulated promoters that are active only in specific organs, tissues or cell types of the organism, and/or sensitive to regulation signals.

Such regulation of promoters can be on different levels and more particularly on the level of specificity of expression (spatial specificity) or on the level of specificity of regulation (temporal specificity). Some promoters are regulated on only one of these levels in a certain tissue or on both levels, for example in a certain tissue under certain circumstance. Even more, one promoter can be regulated only on one level in one tissue and on both levels in another tissue.

Therefore, included within the scope of the term "promoters" are regulated promoters. Examples of regulated promoters are cell-specific promoter sequences, tissue-specific promoter sequences, organ-specific promoter sequences, cell cycle specific gene promoter sequences, inducible promoter sequences and constitutive promoter sequences that have been modified to confer expression in a particular part of the plant at any one time, such as by integration of said constitutive promoter within a transposable genetic element (*Ac*, *Ds*, *Spm*, *En*, or other transposon).

Alternatively and/or additionally, the expression is regulated ubiquitously throughout the whole plant. Alternatively and/or additionally, the expression is constitutive. The skilled craftsman will understand that a "constitutive promoter" is a promoter that is transcriptionally active in an organism, preferably a plant, during most, but not necessarily all phases of its growth and development. Similarly, the skilled craftsman will understand that a "ubiquitous promoter" is a promoter that is transcriptionally active throughout most, but not necessarily all parts of an organism, preferably a plant.

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The strength of a promoter is also an important parameter when selecting a suitable promoter for regulating expression of a gene. Depending on the type of gene and/or the type of effect of expression, one may want to choose a weak or a strong promoter. Accordingly, the present invention relates to the nucleic acids as described above regulating the expression of the operably linked nucleic acid in a weak or in a strong manner. For example a standard of a strong promoter active in plant is CaMV 35S promoter.

The term "cell-specific" shall be taken to indicate that expression is predominantly in a particular cell or cell-type, preferably of plant origin, albeit not necessarily exclusively in said cell or cell-type.

Similarly, the term "tissue-specific" shall be taken to indicate that expression is predominantly in a particular tissue or tissue-type, preferably of plant origin, albeit not necessarily exclusively in said tissue or tissue-type.

Similarly, the term "organ-specific" shall be taken to indicate that expression is predominantly in a particular organ, preferably of plant origin, albeit not necessarily exclusively in said organ.

Those skilled in the art will be aware that an "inducible promoter" is a promoter the transcriptional activity of which is increased or induced in response to a developmental, chemical, environmental, or physical stimulus.

Alternatively or additionally, a promoter can be regulated by differential methylation of its DNA sequences. Different sites of methylation are described and these different sites are each differentially regulated by a set of different enzymes. Again these enzymes are differentially regulated during development of the organism, resulting in a very specific and dynamic mechanism to control promoter activity.

Operably linking a nucleic acid molecule under the regulatory control of a promoter sequence, or in operable connection with a promoter sequence, means positioning said nucleic acid molecule such that expression is controlled by the promoter sequence. A promoter is positioned upstream, or at the 5'-end, and comprises sequences that are in front of the start site of transcription, of the nucleic acid molecule, which it regulates. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control may be defined by the positioning of the element in its natural setting (i.e., the gene from which it is derived). Again, as is known in the art, some variation in this distance can also occur.

Promoters may contain additional copies of one or more specific regulatory elements, to further enhance expression and/or to alter the spatial expression and/or temporal expression of a nucleic

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acid molecule to which it is operably connected. Such regulatory elements may be placed adjacent to regulatory sequences (e.g. regulatory boxes or minimal promoter) of a heterologous promoter, to form a hybrid promoter capable of regulating expression of a nucleic acid molecule and combining the functional properties of its composing fragments. Further, functional fragments of the promoters of the present invention, for example some regulatory boxes or the minimal promoters, can be placed in the background of another promoter to form a hybrid promoter.

Accordingly, the invention further extends to the isolated nucleic acid as mentioned above, wherein the isolated nucleic acid is a hybrid promoter.

In a particular embodiment of the invention, the hybrid promoter comprises a minimal promoter of one promoter and one or more regulatory element(s) of another promoter, at least one of these promoters being a promoter of the present invention. One example of such a hybrid promoter is, the minimal promoter of a strong constitutive promoter, such as CaMV35S promoter, and the regulatory elements of the promoter of the present invention. The minimal promoter, or core promoter is comprising the minimal sequences to promote expression of a gene. For example the minimal promoter comprises a TATA box and a transcription initiation site. Another example of a hybrid promoter according to the present invention is a promoter consisting of fragments of a sequence as given in any of SEQ ID NO 1 to 69 and at least one fragment of a strong constitutive promoter sequence such as a ubiquitin promoter.

A further embodiment of the invention encompasses a method for conferring tissue-specificity, and/or constitutive expression to another promoter sequences, comprising the fusion of a promoter according to the present invention or a fragment thereof, to a second promoter sequence normally not exhibiting said tissue specificity and/or constitutive expression. Such modifications and fusions can be achieved by routine experimentation by those skilled in the art.

Further, the invention encompasses a genetic construct comprising a nucleic acid sequence of the present invention as described above. In one particular embodiment, the invention encompasses a genetic construct, comprising a nucleic sequence as represented by any of SEQ ID NO 1 to 69, or a fragment thereof, said isolated nucleic acid being capable of regulating transcription of an operably linked nucleic acid.

Further, the invention relates to a genetic construct as mentioned above comprising

(a) said nucleic acid sequence capable of regulating transcription of an operably linked nucleic

acid

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(b) an operably linked nucleic acid sequence

(c) a 3' transcription terminator

A genetic construct encompasses a nucleic acid molecule that that has been manipulated or that is made by genetic engineering.

- 5 According to a more particular embodiment of the invention, the genetic construct as mentioned above is a plant expression vector.

With "vector" is meant a DNA sequence which can be introduced in an organism by transformation and can be stably maintained in said organism. Vector maintenance is possible in e.g. cultures of
10 *Escherichia coli*, *A. tumefaciens*, *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe*. Other vectors such as phagemids and cosmid vectors can be maintained and multiplied in bacteria and/or viruses. Vector sequences generally comprise a set of unique sites recognized by restriction enzymes, the multiple cloning site (MCS), wherein one or more non-vector sequence(s) can be inserted. "Expression vectors" form a subset of vectors which, by virtue of comprising the
15 appropriate regulatory sequences enabling the creation of an expressible format for the inserted non-vector sequence(s), thus allowing expression of the protein encoded by said non-vector sequence(s). Expression vectors are known in the art enabling protein expression in organisms including bacteria (e.g. *E. coli*), fungi (e.g. *S. cerevisiae*, *S. pombe*, *Pichia pastoris*), insect cells (e.g. baculoviral expression vectors), animal cells (e.g. COS or CHO cells) and plant cells (e.g.
20 potato virus X-based expression vectors, see e.g. Vance et al. 1998 – WO 98/44097).

Typically, a plant expression vector according to the present invention is to be transformed in plants to achieve a certain effect in the transformed plant, for example the expression of a nucleic acid operably linked to the introduced promoter. Typically, a plant expressible vector according to
25 the present invention, comprises next to the promoter according to the present invention, further elements necessary to successfully achieve transformation and to successfully achieve the objective of introducing the promoter of the present invention into the plant examples of such further elements are a selectable marker, a screenable marker, T-DNA for stable integration of the introduced nucleic acids, origin of replication, operably linked nucleic acids, 5' untranslated
30 regions, 3' untranslated regions comprising 3' transcription terminator etc.

As used herein, the term "selectable marker gene" or "selectable marker" or "marker for selection" includes any gene which confers a phenotype on a cell in which it is expressed to facilitate the identification and/or selection of cells which are transfected or transformed with a gene construct
35 of the invention or a derivative thereof. Suitable selectable marker genes contemplated herein

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include the ampicillin resistance (Amp^r), tetracycline resistance gene (Tc^r), bacterial kanamycin resistance gene (Kan^r), phosphinothricin resistance gene, neomycin phosphotransferase gene (*nptII*), hygromycin resistance gene, β -glucuronidase (GUS) gene, chloramphenicol acetyltransferase (CAT) gene, green fluorescent protein (*gfp*) gene (Haseloff *et al*, 1997), and luciferase gene, amongst others.

"Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. In case the control sequence is a promoter, it is obvious for a skilled person that double-stranded nucleic acid is preferably used. The term "terminator" refers to a DNA sequence at the end of a transcriptional unit which signals termination of transcription. Terminators are 3'-non-translated DNA sequences containing a polyadenylation signal, which facilitates the addition of polyadenylate sequences to the 3'-end of a primary transcript. Terminators active in cells derived from viruses, yeasts, moulds, bacteria, insects, birds, mammals and plants are known and described in the literature. They may be isolated from bacteria, fungi, viruses, animals and/or plants.

Examples of terminators particularly suitable for use in the gene constructs of the present invention include the *Agrobacterium tumefaciens* nopaline synthase (NOS) gene terminator, the *Agrobacterium tumefaciens* octopine synthase (OCS) gene terminator sequence, the Cauliflower mosaic virus (CaMV) 35S gene terminator sequence, the *Oryza sativa* ADP-glucose pyrophosphorylase terminator sequence (t3'Bt2), the *Zea mays* zein gene terminator sequence, the *rbcs-1A* gene terminator, and the *rbcs-3A* gene terminator sequences, amongst others.

Further the present invention encompasses a host cell containing an isolated nucleic acid molecule according to the invention and as described above or a genetic construct as described above. In a particular embodiment, the host a bacterial cell, insect, fungal, plant or animal cell.

According to another embodiment of the invention, there is provided a transgenic plant containing an isolated nucleic acid or a genetic construct as mentioned above.

As stated previously "plant" as used herein means encompasses whole plants, ancestors and progeny of the plants and plant parts, plant cells, tissues and organs. Preferred plants are also described above.

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The invention further extends to harvestable parts of said transgenic plants, such as seeds, leaves, fruits, stem cultures, rhizomes, flowers, roots, tubers and bulbs. Also the invention extends to the progeny derived from any of the plants or plant parts thereof according to the present invention.

Preferably, in the transgenic plant the nucleic acid of the invention is stably integrated into the genome of said plant cell.

The invention also encompasses a method for regulating the expression of a nucleic acid, comprising operably linking said nucleic acid sequence to an isolated nucleic acid as described above.

More specifically, in one embodiment, the invention provides a method for regulating the expression of a nucleic acid, comprising operably linking said nucleic acid sequence to a promoter comprising a nucleic acid sequence as presented in any of SEQ ID NO 1 to SEQ ID NO 69, or a fragment thereof.

Further, according to a preferred embodiment, the expression of the operably linked nucleic acid is regulated in a plant.

Further there is provided a method, wherein the expression of said nucleic acid is regulated in one or more particular cells, tissues or organs of a plant.

According to yet another embodiment, the invention provides a method for the production of a transgenic plant, comprising the introduction into the plant of an isolated nucleic acid or a genetic construction mentioned above.

The invention also relates to a method for the production of a transgenic plant, comprising the transformation of a plant cell or plant tissue and further comprising regenerating a plant from said plant cell or plant tissue. The plants may also be able to grow, or even reach maturity or even to set seeds or even progeny is produced from these seeds or even further, that progeny is fertile. Alternatively or additionally, the transformed and regenerated plants may also produce progeny by non-sexual propagation such as cloning, grafting.

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Accordingly, the invention provides a method for the production of a transgenic plant, comprising introduction into a plant a genetic construct comprising a nucleic acid sequence as presented in any of SEQ ID NO 1 to SEQ ID NO 69, or a fragment thereof.

5 The person skilled in the art will recognize that the provision of the sequences SEQ ID NO 1 to 276, readily makes available the tools to isolate useful promoter with homologous sequence to any of SEQ ID NO 1 to 69 or to isolate promoters from genes that are homologous to any of SEQ ID NO 208 to 276. Therefore the present invention also encompasses a method for isolating a 5' regulatory sequence, capable of regulating expression of an operably linked nucleic acid,
10 comprising the step of screening nucleic acid sequences for sequences that are homologous to any of the sequences represented by SEQ ID NO 1 to 69 or SEQ ID NO 208 to 276.

Further the invention relates to a method as described above, comprising the additional steps chosen from the group consisting of

- 15 (a) Using the identified homologues sequence as described above to screen a genomic library prepared from the organism of origin of said homologous sequence, identifying the transcription initiation site on the identified the genomic DNA and/or
(b) Finding the translation initiation site on the genomic sequence as available in silico, and further designing specific primers for amplification of DNA region 5' upstream of said
20 transcription initiation site.

Also the invention relates to the use of an isolated nucleic acid as mentioned above to regulate the expression of an operably linked nucleic acid.

25 The present invention extends to the identification of regulatory proteins that are involved in the regulation of the activity of the promoters according to the present invention. Such identification can be achieved by using a One hybrid system. In such a one hybrid system the sequences according to any one of SEQ ID NO 1 to 69 are operably linked to the GAL transcription activator and transfected to a yeast cell culture. That yeast cell culture is again transformed with a library of
30 constructs encoding candidate regulatory factors. In the yeast cell, which contains the matching regulatory factor, the promoter will become active and a reporter gene will be transcribed. Subsequently, the coding sequence for the regulatory factor of the promoter can be isolated from the yeast cell and further characterized. The one hybrid system (Li JJ and Herskowitz I. Isolation of ORC6, a component of the yeast origin recognition complex by a one-hybrid system, Science.
35 1993 Dec 17;262(5141):1870-4) is a variant of original two hybrid system described by Chien et

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al. (The two-hybrid system: a method to identify and clone genes for proteins that interact with a protein of interest, Proc Natl Acad Sci U S A. 1991 Nov 1;88(21):9578-82).

According to yet another embodiment of the present invention, there is provided a computer readable medium having stored thereon computer executable instructions for performing a method comprising: a) receiving data on expression in a cell of a plant of a nucleic acid molecule having at least 70% sequence identity to a nucleotide sequence comprising any of SEQ ID NO 208-276; and b) identification of the translation initiation site of these nucleic acid molecules © Identification of the upstream nucleic acid region, comprising the promoter of these nucleic acid molecules. Also there is provided by the present invention a computer-readable medium having stored thereon a data structure comprising sequence data for at least one nucleic acid molecule having at least 70% nucleic acid sequence identity to a nucleotide having a nucleotide sequence as set forth in any of SEQ ID NO. 1 to 276 or a nucleotide sequence complementary thereto; and optionally a module receiving the nucleic acid molecule sequence data, which compares the nucleic acid molecule sequence data to a least one other nucleic acid sequence.

The present invention will now be described with reference to the following figures in which:

Figure 1. shows a schematic representation of a promoter. Regulatory elements (boxes), can be for example responsive for tissue specificity and spatio-temporal activity. The minimal promoter or core promoter, including an eventual TATA box, is the place where the RNA polymerase II is fixed. The transcription initiation element (INR) includes the initiation start. The 5' untranslated region (5'UTR) will be present in the transcribed pre-messenger RNA and eventually in the mRNA but not in the translated protein. The translation initiation codon is presented as ATG.

Figure 2 shows a binary vector, suitable for the recombination cloning of the β -glucuronidase gene under the control of any of the promoters of the present invention and the T-zein - T-rbcS-deltaGA double terminator sequence, via the Gateway recombination cassette. This vector is then suitable for the transformation and the expression of the promoter-GUS cassette in plants. This vector contains a T-DNA derived from the Ti Plasmid, limited by a left border (LB repeat, LB Ti C58) and a right border (RB repeat, RB Ti C58). From the left border to the right border, this T-DNA contains: a 'constitutive promoter – selectable marker gene – terminator' cassette for antibiotic selection of transformed plants; a 'constitutive promoter – screenable marker gene – terminator' cassette for visual screening of transformed plants; the Gateway LR recombination cassette, containing a chloramphenicol resistance gene and the ccdB suicide gene for counter selection of non-recombined plasmids, were the promoter according to the present invention will

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be inserted; the *Escherichia coli* b-glucuronidase coding sequence, interrupted by the second intron of the potato light-inducible tissue-specific ST-LS1 gene and the zein and *rbcS*-deltaGA double terminator'. This vector also contains an origin of replication from pBR322 for bacterial replication and a selectable marker (Spe/SmeR) for bacterial selection with spectinomycin and streptomycin.

Figure 3 shows the results of the GUS staining of plants or plant parts and shows the activity of the promoter PRO0005 as represented by SEQ ID NO 1;

Figure 4 shows the expression pattern of PRO0058 as represented by SEQ ID NO 8;

Figure 5 shows the expression pattern of PRO0061 as represented by SEQ ID NO 9;

Figure 6 shows the expression pattern of PRO0063 as represented by SEQ ID NO 10 calli (A) and plant parts, young leaves, old leaves and seeds of A plants (B) are shown, there is expression in young tissues;

Figure 7 shows the expression pattern of PRO0081 as represented by SEQ ID NO 14, the picture of a positive C plant having expression in the shoot, is shown;

Figure 8 shows the expression pattern of PRO0087 as represented by SEQ ID NO 15, endosperm specific expression is visible;

Figure 9 shows the expression pattern of PRO0095 as represented by SEQ ID NO 17, expression in the embryo of the seed is visible;

Figure 10 shows the expression pattern of PRO0111 as represented by SEQ ID NO 23, , expression in root and more particularly in meristem, is visible;

Figure 11 shows the expression pattern of PRO0116 as represented by SEQ ID NO 24, weak constitutive and/or weak expression in the meristem is visible;

Figure 12 shows the expression pattern of PRO0117 as represented by SEQ ID NO 25, expression in endosperm is visible;

Figure 13 shows the expression pattern of PRO0123 as represented by SEQ ID NO 27, strong shoot specific expression is visible;

Figure 14 shows the expression pattern of PRO0170 as represented by SEQ ID NO 35, strong constitutive expression is visible (A) are B plants, (B) are a old and young leaves and seeds from A plants and (C) are calli;

Figure 15 shows the expression pattern of PRO0171 as represented by SEQ ID NO 36, constitutive expression is visible and

Figure 16 shows the expression pattern of PRO0173 as represented by SEQ ID NO 37, constitutive or shoot specific expression is visible ;

Example 1. Identification and isolation of the promoters

Results of the expression-pattern of these newly isolated promoters are represented in Table 1.

- 5 **Table 1:** list of promoters isolated and characterised in the present invention. The promoter sequences are given a SEQ ID NO and a promoter number (PRO NO). The coding sequence which is controlled by the promoter is annotated by its gene name. A published sequence that contains the sequence of the promoter or that contains the closest variant of the promoter of the present invention is named by its Genbank accession number (gene/BAC, Genbank). Sequences
- 10 for which such a BAC or gene Genbank accession number is not available are cloned via Genome walking and constitute a sequence which is not part of a published sequence. Also the length of the promoter sequence is given. The TC (tentative contig) number is the accession number from the TIGR rice transcribed sequence database (<http://www.tigr.org/tdb/e2k1/osa1/>) The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 20850), that refers
- 15 to the transcribed sequence that is naturally under the control of the corresponding promoter of the present invention. Said transcribed regions can be sequence predictions based on contig building from all known EST, plus known cDNA, plus reconstructed mRNA from gene sequences plus translations of proteins. The TC number, and the sequence deposited hereunder, is useful to position on the genome a gene of interest and Therefore also its promoter.

SEQ ID NO	PRO NO	Gene name	Expression	TC NO	Genbank	length
1	PRO0005	putative beta-amylase	scutellum	TC90358	AC022457	1215
2	PRO0012	lipase (putative)	not detected	TC83463	AP001633	1188
3	PRO0014	transferase (putative)	not detected	TC84853 + TC90672	AP002482	889
4	PRO0016	peptidyl prolyl cis-trans isomerase (putative)	not detected	TC52979	AP000559	1492
5	PRO0019	unknown	not detected	TC53511	AP003105	1217
6	PRO0020	prp protein (putative)	not detected	TC45098	AP001383	1215
7	PRO0029	noduline (putative)	not detected	TC39230	AP002747	1259
8	PRO0058	proteinase inhibitor Rgpl9	seed	TC83117	AF044059	1301
9	PRO0061	beta expansine	weak in	TC89913	AC020666	1243

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		EXPB9	young flowers			
10	PRO0063	structural protein	young tissues + calli + embryo	TC89985	AP001278	1019
11	PRO0069	xylosidase (putative)	not detected	TC48746	AP002538	1144
12	PRO0078	CBP80	to come	TC82961+TC956 21	AAAA01015 493+AAAA0 1017897	1178
13	PRO0079	starch branching enzyme I	not detected	TC52500	D10838	1204
14	PRO0081	putative caffeoyl-CoA 3-O-methyltransferase	shoot	TC89891	AP000364	1212
15	PRO0087	prolamine RM9	strong in endosperm	TC82719	none	1204
16	PRO0092	allergen RA5	to come	TC62788	AP003963	1242
17	PRO0095	putative methionine aminopeptidase	embryo	TC89883+TC842 01 (likely) + TC89883 (likely)	AC027133	1216
18	PRO0098	ras-related GTP binding protein	not detected	TC56737	AP003686	1229
19	PRO0104	beta expansine EXPB1	to come	TC63273	AY039023	1294
20	PRO0105	Glycine rich protein	not detected	TC63395	AC091090	1343
21	PRO0108	metallothionein like protein (putative)	not detected	TC56720	AL731784	1283
22	PRO0109	metallothioneine (putative)	not detected	TC56718	AL731784	1208
23	PRO0111	uclacyanin 3-like protein	weak discriminatio n centre / shoot meristem	TC90434	AJ307662	1237

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24	PRO0116	26S proteasome regulatory particle non-ATPase subunit 11	very weak meristem specific	TC83072	AP000969	1100
25	PRO0117	putative 40S ribosomal protein	weak in endosperm	TC90038	AC090871	1216
26	PRO0122	chlorophyll a/b-binding protein precursor (Cab27)	to come	TC56736	AP004700	1210
27	PRO0123	putative protochlorophyllide reductase	strong leaves	TC89839	AL606456	1179
28	PRO0131	GOS9	not detected	TC61377	X51909	1226
29	PRO0138	cyclin A2	not detected	TC60065	AP002481	1196
30	PRO0139	Cyclin D2	to come	NO TC	AAAA01016424	1252
31	PRO0140	Cyclin D3	to come	NO TC	AL607098	1307
32	PRO0156	HVA22 homologue (putative)	not detected	TC85840	AP002899	1274
33	PRO0157	EL2	to come	TC65908	AC113930	1241
34	PRO0169	aquaporine	to come	TC56634	AP005108	1267
35	PRO0170	High mobility group protein	strong constitutive	TC89846	AP004004	1130
36	PRO0171	reversibly glycosylated protein RGP1	weak constitutive	TC82935	AC090874	1230
37	PRO0173	cytosolic MDH	shoot	TC82977	AC037425	1234
38	PRO0176	CDPK7	to come	TC90377	AL662987	1314
39	PRO0177	Cdc2-1	to come	TC63522	AP004765	1087
40	PRO0197	sucrose synthase 3	not detected	TC56770	AP004988	704
41	PRO0198	OsVP1	not detected	TC64259	AP003436	1121
42	PRO0200	OSH1	to come	TC64419	D16507	1042
43	PRO0208	putative chlorophyllase	to come	NO TC	AC027658	1216
44	PRO0210	OsNRT1	to come	TC91931	AAAA01004	2559

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					908	
45	PRO0211	EXP3	to come	TC65369	AAAA01011 511	1248
46	PRO0216	phosphate transporter OjPT1	to come	TC90666	AC025907	1186
47	PRO0220	RFL	to come	TC96414	AF397034	1221
48	PRO0009	putative cellulose synthase	weak in roots	TC83635	AC022457	1038
49	PRO0075	prolamine 10 Kda	strong in endosperm	TC82859	AF294580	838
50	PRO0076	allergen RA2	strong in endosperm	TC89681+TC896 80	D11434	978
51	PRO0110	RCc3	strong root	TC89946	AC037426	1264
52	PRO0133	chitinase Cht-3	very weak meristem specific	TC85888	D16223	1808
53	PRO0141	cyclophyllin 2	shoot and seed	TC89848	L29469	1139
54	PRO0091	prolamine RP5	strong in endosperm	TC89670	AF156714	1052
55	PRO0001	Metallothionein Mte	scutellum + calli	TC82827	AF048750	883
56	PRO0077	prolamine RP7	strong in endosperm	TC89636	AF194115	688
57	PRO0080	Metallothioneine-like ML2	scutellum + calli	TC82749	AP003197	1258
58	PRO0090	prolamine RP6	strong endosperm	TC89674	X65064	668
59	PRO0126	metallothionein RiCMT	strong discriminatio n centre / shoot meristem	TC82826	AB041032	1245
60	PRO0129	GOS2	strong	TC89792 +	X51910	2195

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			constitutive	TC89796		
61	PRO0135	alpha-globulin	strong in endosperm	TC82833	D50643	1008
62	PRO0136	alanine aminotransferase	weak in endosperm	TC90370	AB007404	1395
63	PRO0146	sucrose synthase SS1 (barley)	medium constitutive	TC27215	X73221	2115
64	PRO0147	trypsin inhibitor ITR1 (barley)	weak in endosperm	TC27238	X65875	342
65	PRO0149	ubiquitin 2 with intron	strong constitutive	TC82803	AF184280	2753
66	PRO0151	WSI18	embryo + stress?	TC84300	AP003023	1828
67	PRO0175	RAB21	embryo + stress?	TC83646	Y00842	1553
68	PRO0218	oleosin 18kd	aleurone + embryo	TC83046	AF019212	1236
69	PRO0219	ubiquitin 2 without intron	to come	TC82803	AF184280	1488

Public domain databases were searched for families of rice expressed sequence tags (ESTs) with interesting distribution among source cDNA libraries. For each family, the number of ESTs represented in each organ was normalized. This procedure is equivalent to an "in silico" Northern-blot, and allowed to identify sequences that are strongly expressed or specific for a particular organ. By sequence homology with annotated sequences in public databases and based on literature searches, these genes were identified and a (putative) function and corresponding name was given. Sequence homology is found using standard computer programs such as Blast N using standard parameters (G Cost to open a gap [Integer] default = 5, E Cost to extend a gap [Integer] default = 2, q Penalty for a mismatch in the blast portion of run [Integer] default = -3, r Reward for a match in the blast portion of run [Integer] default = 1, e Expectation value (E) [Real] default = 10.0, W Word size, default is 11 for blastn, 3 for other programs, v Number of one-line descriptions (V) [Integer] default = 100, b Number of alignments to show (B) [Integer] default = 100, Matrix =BLOSUM62) or by using the program BLASTX using standard parameters (Frameshift penalty = No OOF, Query genetic code = standard (1), Matrix = BLOSUM62). The

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ESTs sequences were positioned on genomic BACs (for example rice BACs) using the result of such a BLAST search and the corresponding coding regions were annotated. Starting from the sequence information of the gene and its location in the genome, the promoter of these genes was isolated as the DNA region spanning about 1.2 kb of sequence upstream the translation initiation codon (i.e. first ATG), this codon excluded. In the case when a large intervening sequence such as an intron, was present in the 5' transcribed non-translated region of the gene, the isolated DNA region was spanning about 1.2 kb plus the length of that intervening sequence. Practically, this was done by PCR amplification of the promoter sequences from genomic DNA of Japonica or exceptionally indica rice plants using specific primers with AttB recombination sites, for cloning into the pDONR201 entry plasmid of the GatewayTM system (Life Technologies) using the "BP reaction". These specific primers used for each of the isolated promoters are herein included as SEQ ID NO 70 to 207 and are listed in Table 2. Conditions for PCR were as follows: 1 cycle of 2 minutes denaturation at 94°C, 35 cycles of 1 minute denaturation at 94°C, 1 minute annealing at 58°C and 2 minutes amplification at 68°C, and 1 cycle of 5 minutes at 68°C. The expected size of the fragment is indicated in table 1. A prominent fragment of about the expected size was isolated from gel and purified using the Zymoclean Gel DNA Recovery Kit (Zymo Research, Orange, California).

The purified PCR fragment was used in a standard GatewayTM BP reaction (Invitrogen) using pDONR201 as a recipient vector. The identity and base pair composition of the insert was confirmed by sequencing. The resulting plasmid was quality tested using restriction digests.

Table 2: Overview of the primers used to isolate the promoters of the present invention.

SEQ ID NO PROM	PRO NO	Gene name	Genbank	fwd primer NO	SEQ ID NO FWD PRM	rev primer NO	SEQ ID NO REV PRM
1	PRO0005	putative beta-amylase	AC022457	prm2768	70	prm2769	71
2	PRO0012	lipase (putative)	AP001633	prm2422	72	prm2423	73
3	PRO0014	transferase (putative)	AP002482	prm2424	74	prm2425	75
4	PRO0016	peptidyl prolyl cis-trans isomerase (putative)	AP000559	prm2851	76	prm2852	77
5	PRO0019	unknown	AP003105	prm2770	78	prm2771	79

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6	PRO0020	prp protein (putative)	AP001383	prm2772	80	prm2773	81
7	PRO0029	noduline (putative)	AP002747	prm2774	82	prm2775	83
8	PRO0058	proteinase inhibitor Rgpi9	AF044059	prm2853	84	prm2854	85
9	PRO0061	beta expansine EXPB9	AC020666	prm2426	86	prm2427	87
10	PRO0063	structural protein	AP001278	prm2855	88	prm2856	89
11	PRO0069	xylosidase (putative)	AP002538	prm2857	90	prm2858	91
12	PRO0078	CBP80	AAAA01015 493+AAAA0 1017897	prm5126	92	prm5127	93
13	PRO0079	starch branching enzyme I	D10838	prm3023	94	prm3024	95
14	PRO0081	putative caffeoyl-CoA 3-O- methyltransferase	AP000364	prm3025	96	prm3026	97
15	PRO0087	prolamine RM9	none	prm3006	98	prm2158	99
16	PRO0092	allergen RA5	AP003963	prm3778	100	prm2163	101
17	PRO0095	putative methionine aminopeptidase	AC027133	prm3061	102	prm3062	103
18	PRO0098	ras-related GTP binding protein	AP003686	prm3779	104	prm2166	105
19	PRO0104	beta expansine EXPB1	AY039023	prm4503	106	prm4504	107
20	PRO0105	Glycine rich protein	AC091090	prm2937	108	prm2938	109
21	PRO0108	metallothionein like protein (putative)	AL731784	prm3270	110	prm2940	111
22	PRO0109	metallothioneine (putative)	AL731784	prm2939	112	prm2940	113
23	PRO0111	uclacyanin 3-like protein	AJ307662	prm3031	114	prm3032	115
24	PRO0116	26S proteasome regulatory particle	AP000969	prm3051	116	prm3052	117

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		non-ATPase subunit 11					
25	PRO0117	putative 40S ribosomal protein	AC090871	prm3592	118	prm3049	119
26	PRO0122	chlorophyll a/b- binding protein presursor (Cab27)	AP004700	prm5131	120	prm2195	121
27	PRO0123	putative protochlorophyllide reductase	AL606456	prm3782	122	prm2197	123
28	PRO0131	GOS9	X51909	prm2840	124	prm2841	125
29	PRO0138	cyclin A2	AP002481	prm3037	126	prm3038	127
30	PRO0139	Cyclin D2	AAAA01016 424	prm5133	128	prm3040	129
31	PRO0140	Cyclin D3	AL607098	prm5166	130	prm3065	131
32	PRO0156	HVA22 homologue (putative)	AP002899	prm3011	132	prm3012	133
33	PRO0157	EL2	AC113930	prm5167	134	prm5168	135
34	PRO0169	aquaporine	AP005108	prm3770	136	prm3771	137
35	PRO0170	High mobility group protein	AP004004	prm3772	138	prm3773	139
36	PRO0171	reversibly glycosylated protein RGP1	AC090874	prm3774	140	prm3775	141
37	PRO0173	cytosolic MDH	AC037425	prm3776	142	prm3777	143
38	PRO0176	CDPK7	AL662987	prm5169	144	prm5170	145
39	PRO0177	Cdc2-1	AP004765	prm5135	146	prm5136	147
40	PRO0197	sucrose synthase 3	AP004988	prm4585	148	prm4586	149
41	PRO0198	OsVP1	AP003436	prm5071	150	prm5072	151
42	PRO0200	OSH1	D16507	prm5075	152	prm5076	153
43	PRO0208	putative chlorophyllase	AC027658	prm5753	154	prm5754	155
44	PRO0210	OsNRT1	AAAA01004	prm6153	156	prm6154	157

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			908				
45	PRO0211	EXP3	AAAA01011 511	prm5175	158	prm5176	159
46	PRO0216	phosphate transporter OjPT1	AC025907	prm5717	160	prm5718	161
47	PRO0220	RFL	AF397034	prm6231	162	prm6232	163
48	PRO0009	putative cellulose synthase	AC022457	prm2420	164	prm2421	165
49	PRO0075	prolamine 10 Kda	AF294580	prm3017	166	prm3018	167
50	PRO0076	allergen RA2	D11434	prm3019	168	prm3020	169
51	PRO0110	RCc3	AC037426	prm3780	170	prm3781	171
52	PRO0133	chitinase Cht-3	D16223	prm2844	172	prm2845	173
53	PRO0141	cyclophyllin 2	L29469	prm2848	174	prm2849	175
54	PRO0091	prolamine RP5	AF156714	prm3029	176	prm3030	177
55	PRO0001	Metallothionein Mte	AF048750	prm2766	178	prm2767	179
56	PRO0077	prolamine RP7	AF194115	prm3021	180	prm3022	181
57	PRO0080	Metallothioneine-like ML2	AP003197	prm3512	182	prm3066	183
58	PRO0090	prolamine RP6	X65064	prm3027	184	prm3028	185
59	PRO0126	metallothionein RiCMT	AB041032	prm3007	186	prm3008	187
60	PRO0129	GOS2	X51910	prm2200	188	prm2419	189
61	PRO0135	alpha-globulin	D50643	prm3033	190	prm3034	191
62	PRO0136	alanine aminotransferase	AB007404	prm2846	192	prm2847	193
63	PRO0146	sucrose synthase SS1 (barley)	X73221	prm2962	194	prm2963	195
64	PRO0147	trypsin inhibitor ITR1 (barley)	X65875	prm3075	196	prm2966	197
65	PRO0149	ubiquitine 2 with intron	AF184280	prm2969	198	prm2970	199
66	PRO0151	WSI18	AP003023	prm2973	200	prm2974	201
67	PRO0175	RAB21	Y00842	prm3800	202	prm3801	203

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68	PRO0218	oleosin 18kd	AF019212	prm5755	204	prm5756	205
69	PRO0219	ubiquitine 2 without intron	AF184280	prm6041	206	prm6042	207

Example 2. Vector construction for transformation with a promoter-GUS cassette

Subsequently the isolated promoter was coupled to a reporter gene and expressed in a host plant. The entry clone was subsequently used in an "LR" reaction with p4581, a destination vector used for rice transformation. This vector contains as functional elements within the T-DNA borders: a plant selectable marker; a GFP expression cassette; and a Gateway cassette intended for LR in vivo recombination with the sequence of interest already cloned in the entry clone (Fig. 2). An *Escherichia coli* beta-glucuronidase coding sequence, interrupted by the second intron of the potato light-inducible tissue-specific ST-LS1 gene, is located downstream of this Gateway cassette.

After the LR recombination step, the resulting expression vector can be transformed into *Agrobacterium* strain LBA4044 and subsequently to rice plants.

Example 3. Transformation of rice with promoter-GUS expression vectors

Calli preparation

Mature dry seeds of the rice japonica cultivar Nipponbare were dehusked. Sterilization was done by incubating for one minute in 70% ethanol, followed by 30 minutes in 0.2% HgCl₂, followed by a 6 times 15 minutes wash with sterile distilled water. The sterile seeds were then germinated on a medium containing 2,4-D (callus induction medium). After incubation in the dark for four weeks, embryogenic, scutellum-derived calli were excised and propagated on the same medium. After two weeks the calli were multiplied or propagated by subculture on the same medium for another 2 weeks. Embryogenic callus pieces were sub-cultured on fresh medium 3 days before co-cultivation (boost of cell division activity).

Agrobacterium preparation

Agrobacterium strain LBA4404 harboring binary T-DNA vectors were used for cocultivation. *Agrobacterium* was inoculated on AB medium with the appropriate antibiotics and cultured for 3 days at 28°C. The bacteria were then collected and suspended in liquid co-cultivation medium to a density (OD₆₀₀) of about 1. The suspension was then transferred to a petri dish and the calli immersed in the suspension for 15 minutes. The callus tissues were then blotted dry on a filter paper and transferred to solidified co-cultivation medium and incubated for 3 days in the dark at 25°C.

Plant regeneration

Co-cultivated callus was grown on 2,4-D-containing medium for 4 weeks in the dark at 28°C in the presence of a suitable concentration of the selective agent. During this period, rapidly growing resistant callus islands develop. After transfer of this material to a regeneration medium and incubation in the light, the embryogenic potential was released and shoots developed in the next four to five weeks. Shoots were excised from the callus and incubated for 2 to 3 weeks on an auxin-containing medium from which they were transferred to soil. Hardened shoots were grown under high humidity and short days in a greenhouse. Seeds were then harvested three to five months after transplanting. The method yielded single locus transformants at a rate of over 50 %.

The transformation method used to transform rice is previously described in more detail by (Aldemita and Hodges, Planta, 199 612-617, 1996; Chan *et al.*, Plant Mol. Biol. 22 (3) 491-506, 1993, Hiei *et al.*, Plant J., 6 (2) 271-282, 1994).

Example 4. Evaluation of the first generation of transgenic plants

Transgenic plant growth

Three T0 transgenic rice plants were generated from each callus during transformation. One was sacrificed for GUS staining at the ~5 cm size (C plants), another one was sacrificed for GUS staining at the ~10 cm size, at the time of transplanting in soil (B plants), and one was kept for seed production (A plants). GUS staining was performed on complete C and B plants. On A plants, GUS staining was performed on leaf pieces, flowers and section of seeds at various developmental stages. A plants were allowed to set seed, that were used after harvest for confirmation of the expression pattern in T1 plants if needed.

GUS staining

The material was covered with 90 % ice-cold acetone and incubated for 30 min at 4 °C. After 3 washes of 5 minutes with Tris buffer [15,76 g Trizma HCl (Sigma T3253) + 2,922 g NaCl in 1 l bidist., adjust pH to 7,0 with NaOH], the material was covered by a Tris/ferricyanate/X-Gluc solution [9,8 ml Tris buffer + 0,2 ml ferricyanate stock (0,33 g Potassium ferricyanate (Sigma P3667) in 10 ml Tris buffer)+ 0,2 ml X-Gluc stock (26,1 mg X-Gluc (Europa Bioproducts ML 113A) in 500 µl DMSO)]. Vacuum infiltration was applied for 15 to 30 minutes. The samples were incubated for up to 16 hours at 37 °C until development of blue color. The samples were washed 3 X 5 minutes with Tris buffer. Chlorophyll was extracted in ethanol series of 50 %, 70 % and 90 % (each for 30 minutes) with refreshments of the 90 % if necessary.

Expression patterns of the promoters of the present invention

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Digital pictures of the stained plants were taken and analyzed by eye. The following paragraphs describe in more detail the observed expression patterns of the promoters of the present invention. It is to be understood that when a certain tissue is not mentioned in the describing paragraph below, no expression in that tissue was observed or the tissue was not analysed. For example, if constitutive expression is observed and the constitutive expression was described for the shoot, it is then to be understood that the constitutive expression is absent in all other analysed tissues other than shoot, for example it is then to be understood that the constitutive expression is absent in the roots. Alternatively, the tissue was not analysed.

Further, when such weak or no expression is detected in a certain tissue for any of the promoters of the present invention, it is possibly an inducible promoter, active under circumstances other than the circumstances of these experiments. Other conditions could be tested to evaluate the promoter activity.

PRO0005 - SEQ ID NO1

1 construct was investigated (OS1365) and 28 calli, 0 C, 24 B plants, 22 A plants were analysed: Occasional expression in calli (7%) was observed. Furthermore occasional weak expression in root (4%) and shoot (12%) of B plants was observed. Expression in the scutellum of embryos (43%) and occasional expression in leaves (5%) of A plants was observed. This promoter is embryo/scutellum specific with some leakiness in other tissues.

PRO0012 - SEQ ID NO2

2 constructs were investigated (OS1443 and OS1454) and 30 calli, 28 C, 28 B plants and 11 A plants were analysed. No expression in any part of any plants or calli was observed. Therefore it is concluded that this promoter is a weak promoter and/or the promoter is expressed below the detection level of the reported gene activity.

PRO0014 - SEQ ID NO3

1 construct was investigated (OS1444) and 24 calli, 23 C, 25 B plants and 23 A plants were analysed. There was a weak expression in calli (33%), but no expression was observed in C plant, in B plants and in A plants. Therefore it was concluded that the promoter is very weak and/or the promoter is expressed below the detection level of the reported gene activity.

PRO0016 - SEQ ID NO4

1 construct was investigated (OS1445) and 19 calli, 18 C, 18 B plants and 17 A plants were analysed. No expression was observed in calli, C, B or A plants. Therefore it was concluded that

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the promoter is very weak and/or the promoter is expressed below the detection level of the reported gene activity.

PRO0019 - SEQ ID NO5

5 1 construct was investigated (OS1366) and 10 calli, 0 C, 0 B plants and 10 A plants were analysed. A weak expression was observed in calli (50%). In A plants, no expression in shoot and weak expression in the scutellum of embryo (30%) was observed. Therefore it was concluded that in certain tissues the promoter is very weak. This promoter could be specific to the scutellum. Alternatively, in some tissues the promoter is expressed below the detection level of the reported
10 gene activity.

PRO0020 - SEQ ID NO6

1 construct was investigated (OS1427) and 9 calli, 8 C, 8 B plants and 6 A plants were analysed. No expression was observed in calli, C, B or A plants. Therefore it was concluded that the
15 promoter is very weak and/or the promoter is expressed below the detection level of the reported gene activity.

PRO0029 - SEQ ID NO 7

1 construct was investigated (OS1367) and 0 calli, 0 C, 12 B plants and 12 A plants were
20 analysed. No expression was observed in B or A plants. Therefore it was concluded that the promoter is very weak and/or the promoter is expressed below the detection level of the reported gene activity.

PRO0058 - SEQ ID NO 8

25 1 construct was investigated (OS1370) and 0 calli, 0 C, 13 B plants and 12 A plants were analysed. No expression was observed in B plants. In A plants, there was no expression in the leaves, but strong expression in endosperm and embryo (58-42%) was observed. Therefore it is concluded that promoter PRO0058 is seed-specific.

PRO0061 - SEQ ID NO 9

30 2 constructs were investigated (OS1441 and OS1460) and 20 calli, 32 C, 32 B plants and 32 A plants were analysed. No expression was observed in C and B plants. In A plants expression in the flowers was observed (44%). More particularly expression in lemma of young spikelets was detected. Therefore it was concluded that the promoter is active in lemma of young spikelet and/or
35 the promoter is lemma –specific.

PRO0063 - SEQ ID NO 10

1 construct was investigated (OS1446) and 13 calli, 13 C, 13 B plants and 12 A plants were analysed. In calli weak expression was detected (92%). In C plants, there was no expression in roots and weak expression in some leaves (46%). In B plants, there was no expression in roots and weak expression in young tillers (78%) or young leaves (54%) but no expression in old leaves. In A plants, there was occasional expression in young leaves (17%) and expression in embryo and scutellum (42%) was observed. Therefore it is concluded that this promoter is active in the above ground tissues, such as leaf, stem and seed. Together with the good expression in calli, these data are demonstrating that the promoter is possibly specific for young developing tissues.

PRO0069 - SEQ ID NO 11

1 construct was investigated (OS1485) and 11 calli, 9 C, 10 B plants and 10 A plants were analysed. No expression was observed in calli, C, B, or A plants. Therefore it was concluded that the promoter is very weak and/or the promoter is expressed below the detection level of the reporter gene activity.

PRO0079 - SEQ ID NO 13

1 construct was investigated (OS1559) and 0 calli, 11 C, 1 B plants and 15 A plants were analysed. No expression was observed C, B, or A plants. Therefore it was concluded that the promoter is very weak and/or the promoter is expressed below the detection level of the reporter gene activity.

PRO0081 - SEQ ID NO - SEQ ID NO 14

1 construct was investigated (OS1419) and 20 calli, 20 C, 20 B plants and 20 A plants were analysed. No expression was observed in Calli. Expression was observed in C plants, more particularly very weak expression in root cylinder (40%) and weak expression in young leaves (80%), fainter in old leaves. Expression was also observed in B plants, more particularly very weak expression in root (25%) and weak expression in young leaves (80%). Further expression was observed in A plants, more particularly expression in young leaves (50%). No expression was observed in old leaves and seeds of A plants. Therefore it was concluded that promoter PRO0081 is preferential for above ground tissue and possibly has some leakage in root.

PRO0087 - SEQ ID NO 15

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1 construct was investigated (OS1450) and 14 calli, 14 C, 14 B plants and 14 A plants were analysed. No expression was observed in calli, C plants or B plants. In A plants no expression was observed in leaves, but strong expression was observed in seed endosperm (64%). Therefore it was concluded that promoter PRO0087 is endosperm specific.

5

PRO0095 - SEQ ID NO 17

1 construct was investigated (OS1423) and 16 calli, 14 C, 14 B plants and 16 A plants were analysed. In calli no expression was observed. In C plants there was some expression in root-tips (36 %) but no expression in other parts was observed. In B plants no expression was observed. In A plants expression was observed in embryo (38%) but no expression in leaves, roots, endosperm. Therefore it is concluded that PRO0095 is a good promoter for embryo-specific expression.

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PRO0098 - SEQ ID NO 18

1 construct was investigated (OS1430) and 7 calli, 7 C, 7 B plants and 8 A plants were analysed. No expression was observed in calli, C, B, or A plants. Therefore it was concluded that the promoter is very weak and/or the promoter is expressed below the detection level of the reporter gene activity.

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PRO0105 - SEQ ID NO 20

1 construct was investigated (OS1459) and 21 calli, 21 C, 21 B plants and 20 A plants were analysed. No expression was observed in calli, C, B, or A plants. Therefore it was concluded that the promoter is very weak and/or the promoter is expressed below the detection level of the reporter gene activity.

20

PRO0108 - SEQ ID NO 21

1 construct was investigated (OS1428) and 10 calli, 8 C, 8 B plants and 9 A plants were analysed. No expression was observed in calli, C, B, or A plants. Therefore it was concluded that the promoter is very weak and/or the promoter is expressed below the detection level of the reporter gene activity.

25

30

PRO0109 - SEQ ID NO 22

2 construct were investigated (OS1439 and OS1483) and 21 calli, 40 C, 40 B plants and 40 A plants were analysed. No expression was observed in calli, C, B, or A plants. Therefore it was

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concluded that the promoter is very weak and/or the promoter is expressed below the detection level of the reporter gene activity.

PRO0111 - SEQ ID NO 23

5 1 construct was investigated (OS1421) and 22 calli, 21 C, 22 B plants and 21 A plants were analysed. In C plants no expression was observed. In B plants very weak expression was observed in discrimination centre and meristems (77%). In A plants no expression was observed. Therefore it was concluded that promoter PRO0111 is a weak meristem / discrimination centre specific promoter.

10

PRO0116 - SEQ ID NO 24

1 construct was investigated (OS1679) and 0 calli, 13 C, 14 B plants and A plants were analysed. In C plants, a weak expression was observed in discrimination centre (38%) and young leaf sheath (77%). In B plants a weak expression was observed in discrimination centre/meristem
15 (71%) and young leaf sheath (21%). In A plants no expression was observed. Promoter PRO0116 is therefore is a weak meristem specific promoter.

PRO0117 - SEQ ID NO 25

1 construct was investigated (OS1425) and 9 calli, 9 C, 9 B plants and 9 A plants were analysed.
20 In calli no expression was observed. In C plants there was occasional weak expression in roots (22%) and in young leaf blade (44%), in B plants there was no expression. In A plants there was a weak expression in endosperm (37%). Therefore promoter PRO117 is weak endosperm specific, with so leakiness in very young leaves.

PRO0123 - SEQ ID NO 27

1 construct was investigated (OS1433) and 21 calli, 18 C, 19 B plants and 18 A plants were analysed. In calli there was no expression. In C plants there was no expression in roots but there was strong expression in shoot (33-68%). In B plants there was occasional expression in root and strong expression in shoot (63-79%). In A plants there was very strong expression in young
30 leaves (73%), and an occasional expression in old leaves (39%), but no expression in seeds. Therefore it is concluded that promoter PRO0123 is strong leaf-specific.

PRO0131 - SEQ ID NO 28

1 construct was investigated (OS1482) and 16 calli, 16 C, 16 B plants and 16 A plants were
35 analysed. No expression was observed in calli, C, B, or A plants. Therefore it was concluded that

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the promoter is very weak and/or the promoter is expressed below the detection level of the reporter gene activity.

PRO0138 - SEQ ID NO 29

5 1 construct was investigated (OS1422) and 14 calli, 14 C, 14 B plants and 14 A plants were analysed. No expression was observed in calli, C, B, or A plants. Therefore it was concluded that the promoter is very weak and/or the promoter is expressed below the detection level of the reporter gene activity.

10 PRO0156 - SEQ ID NO 32

1 construct was investigated (OS1451) and 14 calli, 14 C, 14 B plants and 13 A plants were analysed. No expression was observed in calli, C, B, or A plants. Therefore it was concluded that the promoter is very weak and/or the promoter is expressed below the detection level of the reporter gene activity.

15

PRO0170 - SEQ ID NO 35

1 construct was investigated (OS1434) and 23 calli, 21 C, 21 B plants and 14 A plants were analysed. There was expression in most of the calli (52%). In C plants there was weak expression in roots (51%) and strong expression in young leaves (81%). In B plants there was a strong expression in roots (86%) and strong expression in young leaves (86%). In A plants there was strong expression in young (75%) and old (43%) leaves as well as strong expression in embryo, aleurone, and a weaker expression in endosperm (82%). Therefore promoter PRO170 is a good strong constitutive promoter in rice.

25 PRO0171 - SEQ ID NO 36

1 construct was investigated (OS1762) and 18 calli, 11 C, 13 B plants and no A plants were analysed. In calli there is strong expression (44%), in C plants there is expression in all tissues (27%), and in B plants there is some expression in all tissues (16%), mainly in discrimination centres (46%). Therefore it is concluded that promoter PRO0171 is a constitutive promoter.
30 Possibly promoter PRO0171 is a weak constitutive promoter.

PRO0173 - SEQ ID NO 37

1 construct was investigated (OS1435) and 17 calli, 17 C, 17 B plants and 15 A plants were analysed. In calli there was occasional expression (12%) In C plants there was weak expression in upper parts (24-69%). In B plants there was weak expression in young leaves (41%). In A plants

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there was expression in leaves (33%) and strong expression in seeds (38%)? No activity of the promoter could be detected in the root. Therefore it is concluded that the promoter 0173 is active in the above ground tissues. Further promoter 0173 is constitutively active in the shoot.

5 PRO0197 - SEQ ID NO 40

1 construct investigated (OS1437) was investigated and 18 calli, 15 C plants, 15 B plants and 16 A plants. No expression was observed in calli, C, B or A plants. Therefore it is concluded that promoter PRO0197 is very weak and/or the promoter is expressed below the detection level of the reporter gene activity.

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PRO198 - SEQ ID NO 41

1 construct was investigated (OS1557) and 0 calli, 14 C plants, 14 B plants and 14 A plants were analysed. In C plants there was no expression. In B plants there was occasional very weak expression in discrimination centre (29%). In A plants there was occasional very weak expression
15 In seeds (21%). Therefore it is concluded that promoter PRO0198 is very weak and/or the promoter is expressed below the detection level of the reporter gene activity.

PRO 0009 - SEQ ID NO 48

1 construct was investigated (OS1461) and 20 calli, 20 C, 20 B plants and 20 A plants were
20 analysed. In calli there was occasional expression (20 %). In C plants there was a weak expression in root (55%) and an occasional expression in young leaves (10%). In B plants there was weak expression in root (25%) and no expression in leaves. In A plants there was no expression in leaves. Therefore this promoter is a weak root specific promoter with slight leakiness in leaves.

25

PRO 0075 - SEQ ID NO 49

2 constructs were investigated (OS1453 + OS1685) 43 calli, 41 C, 43 B plants and 32 A plants were analysed. Occasional weak expression was observed in calli (33%). Weak expression was observed in discrimination centre/meristem of C plants (56%) and in young leave sheath (46%).
30 Occasional weak expression was observed in young leave blade of B plants (21%). In A plant no expression in leaves and strong expression was observed in endosperm (42%). Therefore this promoter is an endosperm specific promoter, with some leakiness in the leaves.

PRO 0076 - SEQ ID NO 50

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1 construct was investigated (OS1418) and 20 calli, 18 C, 18 B plants and 19 A plants were analysed. Expression was not detected in calli and in C plants. In B plants very weak expression in young leaves (89%) was observed. In A plants no expression in leaves but a strong expression in seed endosperm (68%) was observed. In was therefore concluded that promoter PRO0076 is a strong promoter in endosperm, with leakiness in the leaves.

PRO0110 - SEQ ID NO 51

1 construct investigated (OS1432) was investigated and 25 calli, 14 C, 21 B plants and 21 A plants were analysed. There was no expression in calli, but a strong expression in roots (93%) of C plants and a strong expression in roots (81%) of B plants. A plants were investigated only in the shoots. In the analysed tissues of A plants no expression was observed. It was concluded therefore that promoter PRO0110 is a strong root specific promoter.

PRO0133 - SEQ ID NO 52

1 construct was investigated (OS1687) 15 calli, 12 C, 16 B plants and 12 A plants were analysed. There was a weak expression in calli (66%), but no expression observed in C plants. In B plants a weak expression in discrimination centre/meristem (50%) was observed. No expression was observed in A plants. Based on these results it was concluded that promoter PRO0133 is a weak promoter in meristems and dividing cells.

PRO0141 - SEQ ID NO 53

1 construct was investigated (OS1369) and 0 calli, 0 C, 13 B plants and 12 A plants were analysed. In B plants weak expression in young leaves (69%) was observed and no expression in old leaves or in roots. In A plants expression in leaves (75%) was observed and expression in embryo and endosperm (58-67%). Based on these results it was concluded that promoter PRO0141 is a constitutive promoter in shoot, especially this promoter is active in seed.

PRO0091 - SEQ ID NO 54

1 construct was investigated (OS1558) and 0 calli, 12 C, 12 B plants and 12 A plants were analysed. The C plants showed weak expression in discrimination centre (50%). B plants showed weak expression in discrimination centre (58%). A plants showed strong expression in endosperm (55%) and no expression in leaves. This promoter is useful for strong expression in endosperm, with leakiness in discrimination centre/meristems.

PRO0001 - SEQ ID NO 55

1 construct was investigated (OS1364) and 10 calli, 0 C, 8 B plants and 8 A plants were analysed. In calli strong expression (100%) was observed. In B plants very weak expression in young tillers (38%) was observed and no expression in old leaves or in roots. In A plants occasional expression leaves (13%) was observed and strong expression in embryo, especially in the scutellum (88%). Further in A plants, no expression in endosperm was observed. Therefore it was concluded that this promoters is weak in young aerial tissues and is presumably embryo/scutellum-specific.

PRO0077 - SEQ ID NO 56

1 construct investigated (OS1560) and 0 calli, 11 C, 12 B and 12 A plants were analysed. No expression was observed in C and B plants. In A plants no expression was observed in leaves but there was expression in endosperm (83%). Therefore, this promoter is useful for endosperm specific expression.

PRO0080 - SEQ ID NO 57

1 construct was investigated (OS1429) and 13 calli, 11 C, 12 B plants and 13 A plants were analysed. In calli there was a strong expression (62%). In C plants there was occasional very weak expression in young leaves (33%). In B plants there was occasional very weak expression in young leaves (33%). In A plants there was no expression observed in leaves, but there was a strong expression in scutellum (54%). Therefore, it is concluded that this promoter is embryo/scutellum specific.

PRO0090 - SEQ ID NO 58

12 constructs were investigated (OS0224, OS1329, OS1330, OS1331, OS1333, OS1334, OS1335, OS1420, OS1591, OS1668, OS1700, OS1665) and 24 calli, 24 C, 24 B plants and 194 A plants were analysed. In calli no expression was observed. In C plants there was occasional very weak expression in shoot (17%). In B plants there was occasionally very weak expression in shoot meristems/discrimination centre (25%). In A plants there was strong expression in endosperm (83%) and aleurone (79%), but no or weak expression in embryo and no expression in very early seeds could be observed. It is concluded that promoter PRO0090 is a good promoter for strong expression in endosperm and aleurone with some leakiness in meristems and shoots.

PRO123 - SEQ ID NO 59

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1 construct was investigated (OS1433) and 21 calli, 18 C, 19 B plants and 18 A plants were analysed. In calli no expression could be observed. In C plants no expression in roots was observed, but there was strong expression in shoot (33-68%). In B plants there was occasional expression in root and strong expression in shoot (63-79%). In A plants there was very strong expression in young leaves (73%), occasional expression in old leaves (39%) and no expression in seeds was observed. Therefore, this promoter can be used as a strong leave-specific promoter.

PRO126 - SEQ ID NO 59

1 construct was investigated (OS1532) and 24 calli, 12 C, 12 B plants and 13 A plants were analysed. In calli there was strong expression of the promoter (46%). In C plants no expression was observed in roots, but there was a weak expression in discrimination centre (92%) and in leaves sheath (92%). In B plants there was a weak expression in discrimination centre (100%) corresponding to a strong expression in meristems (100%). There was also weak expression in young leaf sheath (67%) an occasional expression in root (8%) and shoots. In A plants no expression was observed. Therefore this promoter is meristem/calli specific.

PRO0129 - SEQ ID NO 60 – GOS 2

6 constructs were investigated (OS0550, OS1201, OS1348, OS1447 OS1580 OS1699). 37 calli, 59 C, 30 B plants and 69 A plants were analysed. There was strong expression in calli (89%), strong expression in all parts of most of the C and B plants (>50%). In A plants there was low or no expression in old leaf blades, but strong expression in the young leaves (65%) and in the seeds (57%). Thus it was concluded that this promoter is a strong constitutive promoter.

PRO0135 - SEQ ID NO 61

1 construct was investigated (OS1452) and 6 calli, 5 C, 5 B and 6 A plants were analysed. No expression was observed in calli, C and B plants. In A plants no expression in leaves was observed, but there was occasionally very strong expression in endosperm (17%). This promoter is seed/endosperm specific.

PRO0136 - SEQ ID NO 62

1 construct was investigated (OS1368) and 0 calli, 0 C, 16 B plants and 16 A plants were analysed. Occasional expression was observed in young leaves of B plants (6%). In A plants there was a strong expression in endosperm (31%). This promoter is a seed/endosperm specific promoter.

PRO0146 - SEQ ID NO 63 (sucrose synthetase SS1 from Barley)

1 construct was investigated (OS1484) and 7 calli, 7 C, 7 B plants and 7 A plants were analysed. In calli there was a strong expression (71%), in C plants there was expression in shoot tissues (57%) and some expression in root tips (43%). In B plants expression in young leaves (57%) was observed but no expression in old leaves or in root. In A plants there was expression in young leaves (67%), occasionally in old leaves (17%) and a very strong expression in aleurone and endosperm (50%) was observed. This promoter is a constitutive promoter and is especially strong in seed and shoot.

PRO0147 - SEQ ID NO 64

1 construct was investigated (OS1426) and 11 calli, 9 C, 9 B plants and 11 A plants were analysed. There was no expression observed in calli, C and B plants. In A plants there is weak expression in endosperm (50%). This promoter is a weak endosperm specific promoter.

PRO0149 - SEQ ID NO 65

1 construct was investigated (OS1457) and 24 calli, 20 C, 21 B plants and 21 A plants were analysed. There was strong expression in calli (92%) and strong expression in all tissues of most C plants (55%). In B plants strong expression in roots (81%) and young leaves (76%) was observed, but no expression in old leaves. In A plants very strong expression in leaves (62-72%) and very strong expression in seeds (65%) was observed. This promoter is a good strong constitutive promoter.

PRO0151 - SEQ ID NO 66

1 construct was investigated (OS1458) and 22 calli, 16 C, 16 B plants and 13 A plants were analysed. There was strong expression in calli (91%) and weak expression in shoot of C plants (62%). In B plants no expression was observed. In A plants no expression in leaves was observed, but there was very strong expression in aleurone and embryo (46%). Therefore it was concluded that promoter PRO0151 is a strong promoter in calli and in seed, more particularly in the aleurone layer and in the embryo.

PRO0175 - SEQ ID NO 67

1 construct was investigated (OS1436) and 16 calli, 12 C, 15 B plants and 15 A plants were analysed. There was expression in some the calli (31%). In C plants there was expression in discrimination centre (42%) and expression in young leaves (25-58%). In B plants no expression was observed. In A plants there was an occasional expression in young leaves (15 %), and no

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observation of expression in flowers. In A plants there was a very strong expression in aleurone and embryo (60%). This promoters seems to be naturally expressed in developing/maturing seeds, especially in embryo/aleurone. Therefore it was concluded that promoter can be used as a strong promoter in calli and in seed, more particularly in the aleurone layer and in the embryo.

PRO0218 - SEQ ID NO 68

1 construct was investigated (OS0225) and 0 calli, 4 C, 4 B and 4 A plants were analysed. In C plants, weak expression was detected in shoot (50 %). In B plant, no expression was detected. In A plants , there was no expression in leaves, and a strong expression was detected in aleurone (100%), with weaker expression in embryo. No expresison was observed in endosperm. This promoters is a good aleurone /embryo specific promoter.

From the evaluation data it is clear that there was a variation between the different transformed plants (different plants each transformed with the genetic construct comprising the promoter – GUS cassette). It is well known to persons skilled in the art, that the expression of transgenes in plants, and hence also the phenotypical effect due to expression of such transgene, can differ dramatically among different independently obtained transgenic plant or transformation events, and progeny thereof. The transgenes present in different independently obtained transgenic plants or transformation events, differ from each other by the chromosomal insertion locus as well as by the number of transgene copies inserted in that locus and the configuration of those transgene copies in that locus. Differences in expression levels can be ascribed to influence from the chromosomal context of the transgene (the so-called position effect) or from silencing mechanisms triggered by certain transgene configurations (e.g. inwards facing tandem insertions of transgenes are prone to silencing at the transcriptional or post-transcriptional level). The exact configuration and insertion loci of the different transformation events have not yet been determined, and expression levels have not been measured.

Stability of the activity of the promoters of the present invention

The above-mentioned analysis were performed on To plants immediately originating from the transformed calli. The stability of the promoter activity in the next generations or progeny plants of the original T0 plant, the so-called T1 and T2 plants, is now being evaluated.

The T1 plant transformed with promoter170 – GUS were already evaluated. The seeds of the To plants transformed with prom0170-GUS were harvested and these T1 seeds were sowed. The C plants were already evaluated and showed exactly the same expression pattern as the C plants of the T0 generation.

The promoter PRO0090 was studied in T0 plants, T1 seeds, T1 plants and T2 seeds. In all the tissues (including seeds and seed tissues) of these following generations, the seed-specific expression pattern, similar to the expression pattern reported herein, was observed.

5

In conclusion, it has been demonstrated for promoter 170 and promoter PRO0090, that the expression pattern of the promoter in the original transformed plants is stably inherited in plants of subsequent generations. It is expected that the activity of the other promoters of the present invention are also stably inherited in plants of further generations. This feature considerably increased the value of the promoters of the present invention for their use in plant genetic engineering.

10

Stability of the expression pattern of the promoters of the present invention in Arabidopsis thaliana.

15

The above mentioned plant analysis were performed on rice plants. This choice was based on the practical consideration that plant genetic engineering is most profitable for crop plants. However, for many other purposes such as research and horticulture, (small) herbs are being genetically modified, involving the use of particular promoter. Therefore the activity of the promoters of the present invention is being analysed and the stability of the expression pattern among different plant species is being demonstrated. The constructs comprising the expression cassettes promoter-GUS, for example promoter0170 – GUS, are introduced in *Arabidopsis thaliana* and C, B and A plant are being evaluated as mentioned above.

20

25

Accordingly, the promoter PRO0129 was studied in *Arabidopsis thaliana* as a promoter-GUS fusion, and strong constitutive expression was observed in all plant tissues (data not shown). Therefore the regulation of this promoter is conserved in monocots and dicots and the promoter has a stable expression pattern in different types of plant.

Claims

1. An isolated nucleic acid, capable of regulating transcription of an operably linked nucleic acid, comprising a sequence or a fragment thereof, said sequence being selected from the group consisting of:

(a) a nucleic acid comprising the DNA sequence as given in any of SEQ ID NOs 1 to 69 or the complement thereof,

(b) a nucleic acid specifically hybridizing with the nucleotide sequence as defined in (a)

(c) a nucleic acid which is diverging, due to the differences between alleles, to a nucleotide sequence as defined in (a) or (b),

(d) a nucleic acid as defined in any one of (a) to (c), said nucleic acid interrupted by intervening DNA,

(e) a nucleic acid, which is obtainable from plant DNA, said plant DNA comprising a plant gene, which plant gene hybridizes specifically with a sequence as represented by any one of SEQ ID NO 208 to 276 ;

(f) a nucleic acid, which is obtainable from plant DNA, said plant DNA comprising a plant gene, which plant gene is homologous to a sequence as represented by any one of SEQ ID NO 208 to 276, preferably said plant gene is 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% homologous;

(g) a nucleic acid, which is obtainable from plant DNA, said plant DNA comprising a plant gene, which plant gene is encoding a protein which is homologous to a protein encoded by any one of the sequences represented by SEQ ID NO 208 to 276, preferably said plant protein is 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% homologous;

provided that said isolated nucleic acid is not one of the nucleic acids as deposited in the Genbank database under any of the Genbank Accession numbers which are listed in Table 1 or any other publicly available sequence that is not capable of regulating the expression of an operably linked nucleic acid..

2. An isolated nucleic acid according to claim 1, capable of regulating transcription of an operably linked nucleic acid in a plant.

3. An isolated nucleic acid according to claim 1 or 2, capable of regulating transcription of an operably linked DNA sequence in one or more particular cells, tissues or organs of a plant.

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4. The isolated nucleic acid according to any of claim 1 to 3, wherein the isolated nucleic acid is a promoter
5. The isolated nucleic acid according to any of claim 1 to 4, wherein the isolated nucleic acid is a hybrid promoter
6. A genetic construct comprising a nucleic acid sequence according to any of claims 1 to 6.
7. A genetic construct, comprising a nucleic sequence as represented by any of SEQ ID NO 1 to 69, or a fragment thereof, said isolated nucleic acid being capable of regulating transcription of an operably linked nucleic acid.
8. A genetic construct according to any of claims 7 or 8 comprising
- (a) said nucleic acid sequence capable of regulating transcription of an operably linked nucleic acid
 - (b) an operably linked nucleic acid sequence
 - (c) a 3' transcription terminator
9. A genetic construct according to any of claim 6 to 8, which is a plant expression vector.
10. A host cell containing an isolated nucleic acid molecule according to any of claim 1 to 5 or a genetic construct according to any of claims 6 to 9.
11. The host cell according to claim 10, wherein said host cell is a bacterial cell, insect, fungal, plant or animal cell.
12. A transgenic plant containing an isolated nucleic acid according to any of claim 1 to 7 or a genetic construct according to any of claims 10 to 12.
13. Method for regulating the expression of a nucleic acid, comprising operably linking said nucleic acid sequence to an isolated nucleic acid according to any one or more of claims 1 to 6.
14. Method for regulating the expression of a nucleic acid, comprising operably linking said nucleic acid sequence to a promoter comprising a nucleic acid sequence as presented in any of SEQ ID NO 1 to SEQ ID NO 69, or a fragment thereof.

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15. Method according to claim 13 or 14, wherein the expression of said nucleic acid is regulated in a plant.

5 16. Method according to any of claim 13 to 15, wherein the expression of said nucleic acid is regulated in one or more particular cells, tissues or organs of a plant.

10 17. Method for the production of a transgenic plant, comprising the introduction into the plant of an isolated nucleic acid according to any of claim 1 to 7 or a genetic construct according to any of claims 10 to 12.

18. Method for the production of a transgenic plant, comprising introduction into a plant a genetic construct comprising a nucleic acid sequence as presented in any of SEQ ID NO 1 to SEQ ID NO 69, or a fragment thereof.

15 19. Method for isolating a 5' regulatory sequence, capable of regulating expression of an operably linked nucleic acid, comprising the step of screening nucleic acid sequences for sequences that are homologous to any of the sequences represented by SEQ ID NO 1 to 69 or SEQ ID NO 208 to 276.

20 20. Method according to claim 19, comprising the additional steps chosen from the group consisting of

(a) Using the homologues sequence according to claim 19 to screen a genomic library prepared from the organism of origin of said homologous sequence, identifying the transcription initiation site on the identified genomic DNA, and/or

25 (b) Finding the translation initiation site on the available genomic sequence as available in silico,
and designing specific primers for amplification of DNA region 5' upstream of said transcription initiation site.

30 21. Use of an isolated nucleic acid according to any of claims 1 to 5 to regulate the expression of an operably linked nucleic acid.

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22. . Use of a an isolated nucleic acid to regulate the expression of an operably linked nucleic acid, said isolated nucleic acid having a sequence as presented in any one of SEQ ID NO 1 to SEQ ID NO 69 or a fragment thereof.

Abstract

Plant promoters

- 5 The present invention relates to gene expression in plants and provides several nucleic acids suitable for controlling the expression of a gene in one or more particular tissues or organs, and/or during certain developmental stages of the plant, and/or during certain environmental conditions and/or throughout the whole plant.

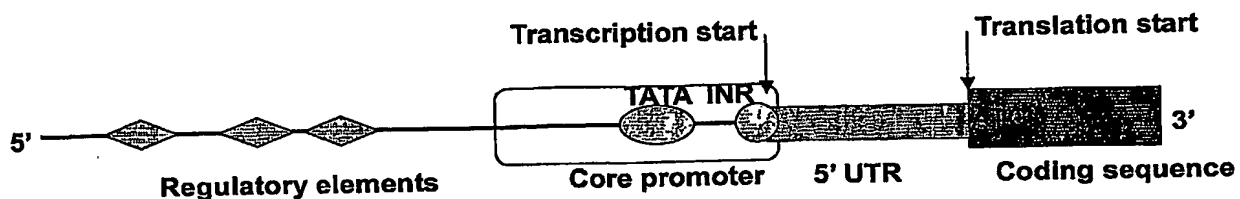


FIGURE 1

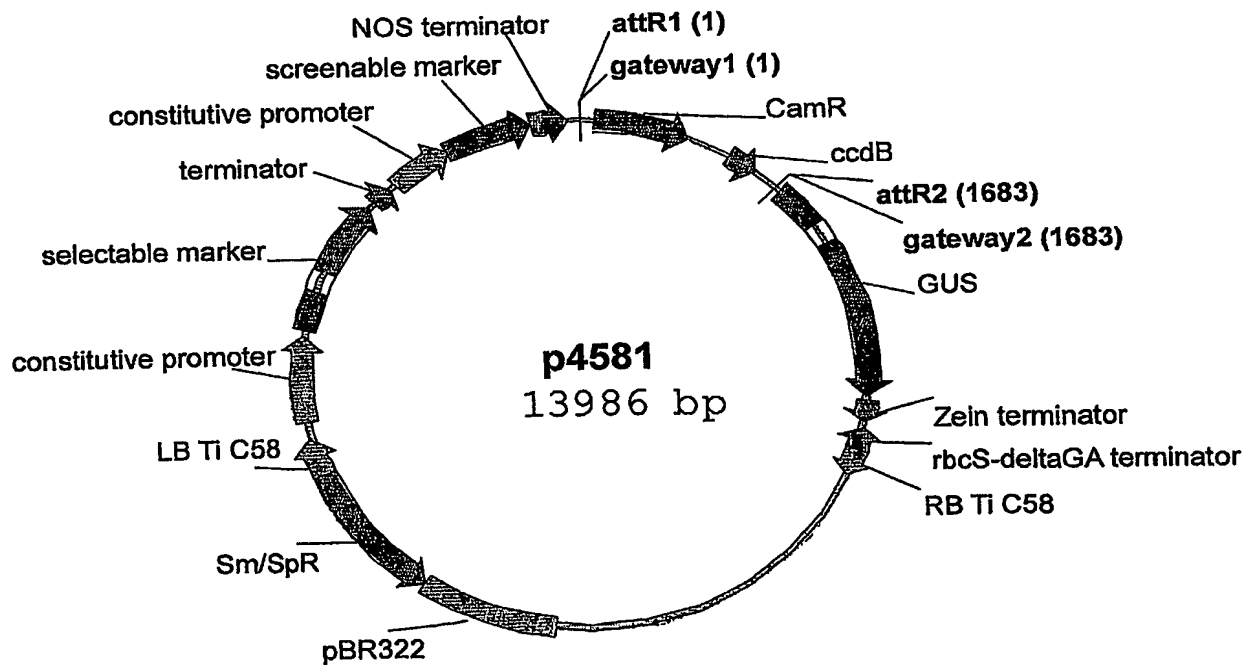


FIGURE 2

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PRO 0005



FIGURE 3

PRO 0058



FIGURE 4

PRO 0061

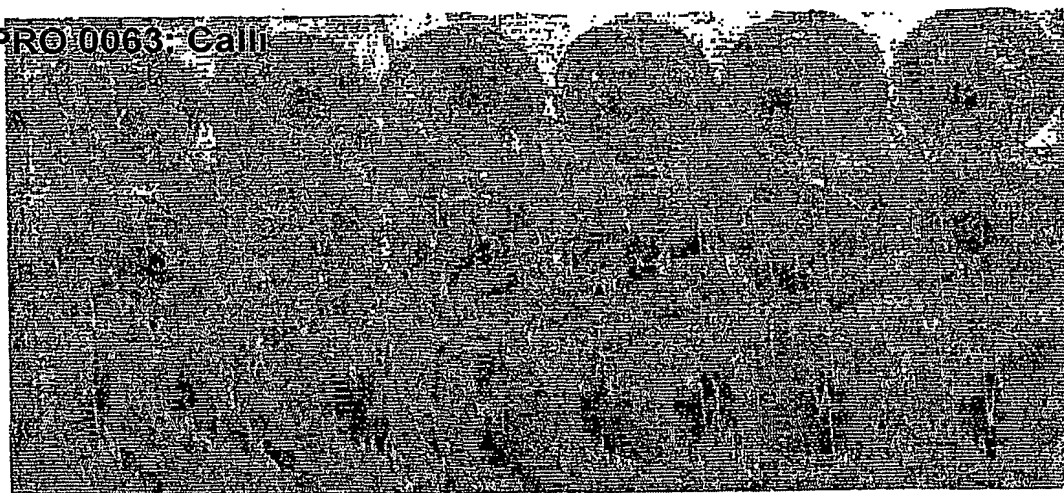


FIGURE 5

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317

A PRO 0063: Calli



B PRO 0063: A plants

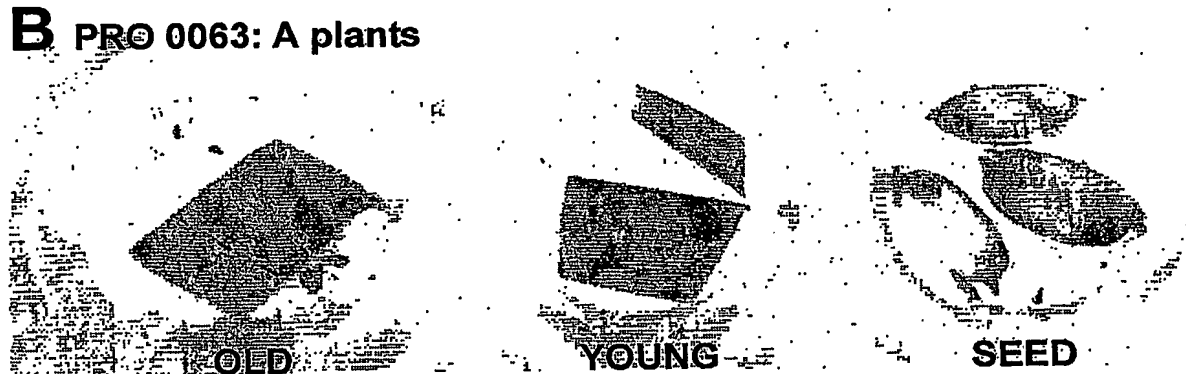


FIGURE 6

PRO 0081



FIGURE 7

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PRO 0087



OLD

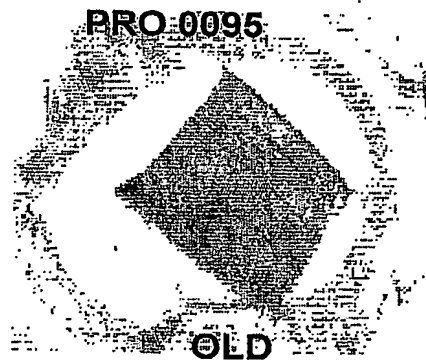
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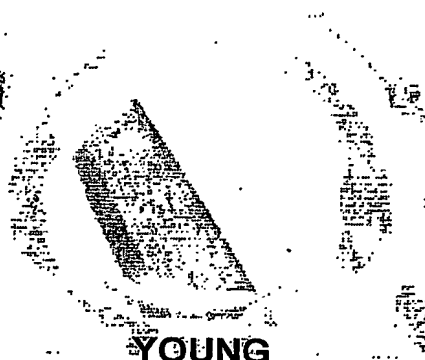
SEED

FIGURE 8

PRO 0095



OLD



YOUNG



SEED

FIGURE 9

PRO 0111

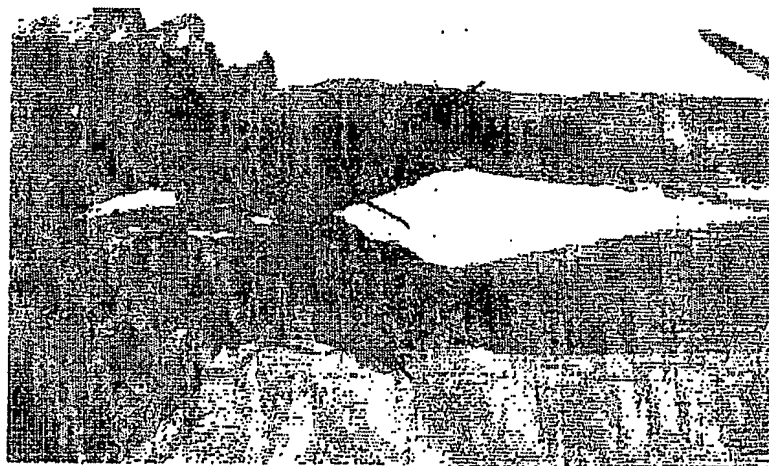


FIGURE 10

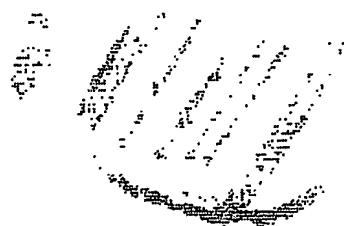
5/7

PRO 0116



FIGURE 11

PRO 0117



OLD

YOUNG



SEED

FIGURE 12

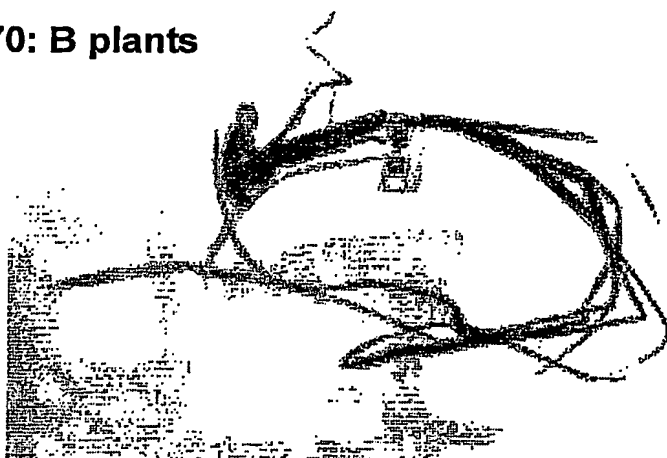
PRO 0123



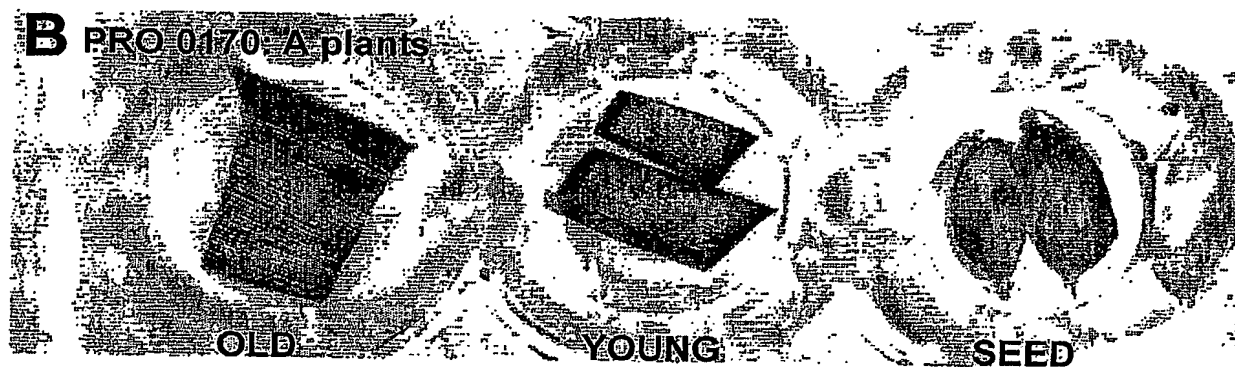
FIGURE 13

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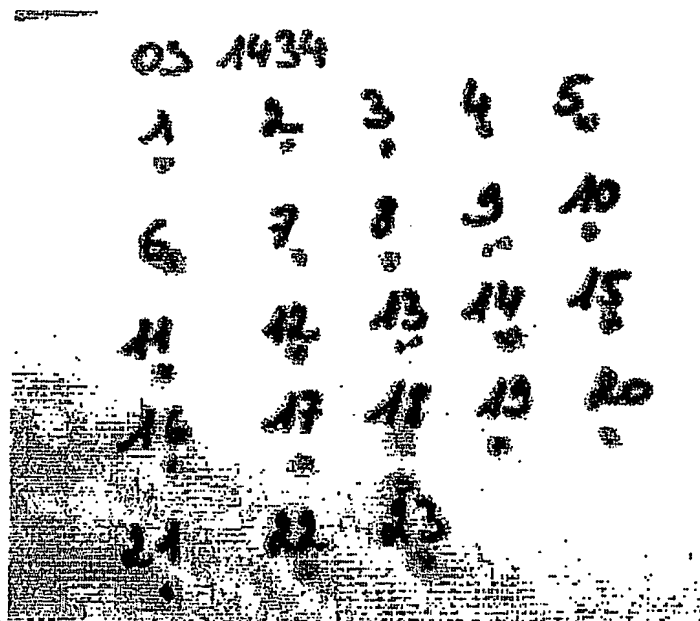
A PRO 0170: B plants



B PRO 0170: A plants



C



PRO 0170: Calli

FIGURE 14

PRO 0171

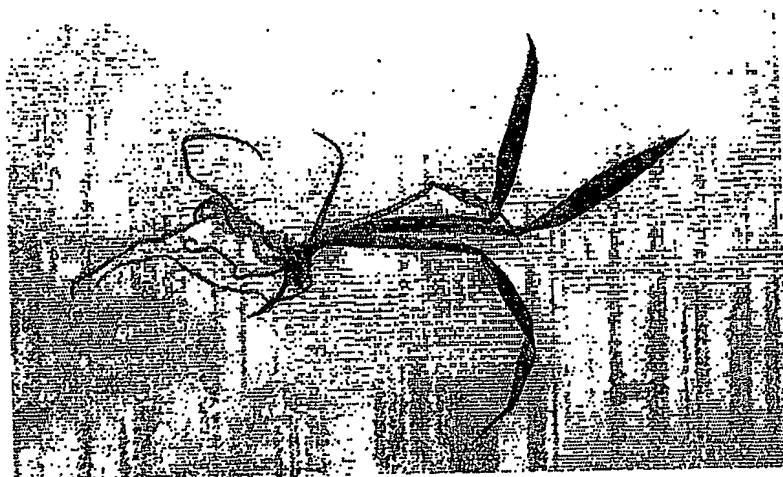
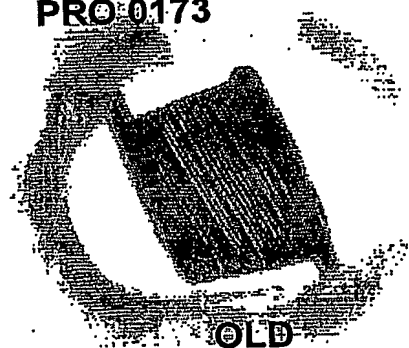


FIGURE 15

PRO 0173



YOUNG

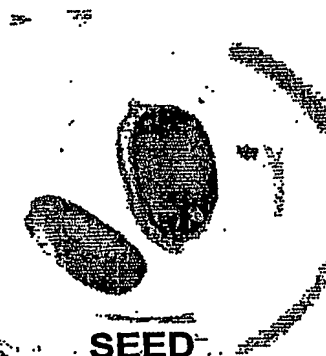


FIGURE 16

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gtgccgtccc ctctcctggc gtcccttcgg gtctcgccct cctcctcgct gctcgteccac 1200
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<210> 7

<211> 1259

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0029 - noduline (putative)

<400> 7

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<210> 8

<211> 1301

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0058 - proteinase inhibitor Rgp19

<400> 8

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cgacgtgaca catggcacgt ccttggagct ttagacatat cgaatctgag cactggagtg	180
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aaaaaaagta gtgtgttctt gagcctatat aagagagggt cacacgctcc agtcgactca	1260

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1301

<210> 9

<211> 1243

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PR00061 - beta-expansine EXPB9

<400> 9

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<210> 10

<211> 1019

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0063 - structural protein

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gatgctaagt ccttcattag tagtatccca catttaatca cagttggaca cacaaaaaaaa	180
aaggcaatgc cattaatatg ccatctctct tgttttccat tgcctaccaa gtgccatatg	240
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<210> 11

<211> 1144

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PR00069 - xylosidase (putative)

<400> 11

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<210> 12

<211> 1178

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0078 - CBP80

<400> 12

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<210> 13

<211> 1204

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0079 - starch branching enzyme I

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<210> 14

<211> 1212

<212> DNA

<213> *Oryza sativa*

<220>

<221> misc_feature

<223> PRO0081 - putative caffeinoyl-CoA 3-O-methyltransferase

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<210> 15

<211> 1204

<212> DNA

<213> *Oryza sativa*

<220>

<221> misc_feature

<223> PRO0087 - prolamine RM9

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<210> 16

<211> 1242

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0092 - allergen RA5

<400> 16

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071-prom-prov.ST25.txt

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<210> 17

<211> 1216

<212> DNA

<213> *Oryza sativa*

<220>

<221> misc_feature

<223> PR00095 - putative methionine aminopeptidase

<400> 17

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<211> 1229

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0098 - ras-related GTP binding protein

<400> 18

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atggtttcta ttgcttgagt ctogaatttc tacattttta atgtatgtta tcaaacaaca	180
tatttttgtt gatttatgtg gaccacagga ctcaacaaca aggtgcatt tatttaagca	240

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<210> 19

<211> 1294

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0104 - beta-expnasin EXPB1

<400> 19

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<210> 20

<211> 1343

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PR00105 - glycine-rich protein

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<210> 21

<211> 1283

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0108 - metallothionein-like protein (putative)

<400> 21

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taaaggcaac gtatttcaaa ttagttaat acatgaacat gttcagagca ggtttgattt	180
ggtcatagaa tcatcaaact caaatgcagt ctagctcatg cattaaattt aaattgaatt	240

071-prom-prov.ST25.txt

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<210> 22

<211> 1208

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0109 - metallothioneine (putative)

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aagaaacatc agtttatatt tcatttcgtg taggacctat caatccaatt cgtacaagag 180
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<210> 23

<211> 1237

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0111 - uclacyanin 3-like protein

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<210> 24

<211> 1100

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0116 - 26S proteasome regulatory particle non-ATPase subunit 1

<400> 24

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<210> 25

<211> 1216

<212> DNA

<213> *Oryza sativa*

<220>

<221> misc_feature

<223> PRO0117 - putative 40S ribosomal protein

<400> 25

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<210> 26

<211> 1210

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0122 - chlorophyl a/b binding protein precursor (Cab27)

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071-prom-prov.ST25.txt

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<210> 27

<211> 1179

<212> DNA

<213> *Oryza sativa*

<220>

<221> misc_feature

<223> PRO0123 - putative protochlorophyllide reductase

<400> 27

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071-prom-prov.ST25.txt

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<210> 28

<211> 1226

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0131 - GOS9

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<210> 29

<211> 1196

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PR00138 - cyclin A2

<400> 29

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tatggagtaa cagagtaacg ccttcaatct ttaaaacacg tcgttttgca caaagttcca	240
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<210> 30

<211> 1252

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0139 - cyclin D2

<400> 30

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<210> 31

<211> 1307

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0140 - cyclin D3

<400> 31

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<210> 32

<211> 1274

<212> DNA

<213> *Oryza sativa*

<220>

<221> misc_feature

<223> PR00156 - HVA22 homologue (putative)

<400> 32

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071-prom-prov.ST25.txt

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<211> 1241

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0157 - EL2

<400> 33

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<210> 34

<211> 1267

<212> DNA

<213> Oryza sativa

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<221> misc_feature

<223> PR00169 - aquaporine

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<211> 1130

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0170 - High Mobility Group protein

<400> 35

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<210> 36

<211> 1230

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0171 - reversibly glycosylated protein RGP1

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1230

<210> 37

<211> 1234

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0173 - cytosolic MDH

<400> 37

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<210> 38

<211> 1314

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0176 - CDPK7

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<210> 39

<211> 1087

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0177 - cdc2-1

<400> 39

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aagcgaaaaa ccccgcttac ccgccccatt tacatcccta cttggatcct gcatgtcagt	240
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<210> 40

<211> 704

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0197 - sucrose synthase 3

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<210> 41

<211> 1121

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0198 - OsVP1

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<210> 42

<211> 1042

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0200 - OSH1

<400> 42

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<210> 43

<211> 1216

<212> DNA

<213> *Oryza sativa*

<220>

<221> misc_feature

<223> PRO0208 - putative chlorophyllase

<400> 43

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<210> 44

<211> 2559

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PR00210 - OsNRT1

<400> 44

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<211> 1248

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0211 - EXP3

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<210> 46

<211> 1186

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0216 - phosphate transporter OjPT1

<400> 46

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gatcaggtat attcctagga tcaaaccata gtacaaataa ccaaatacta caattttaga      480
ttatttgggc agttaggaat aataattttc agtgtccttt gcaaaacott agctccaagc      540
tcattaacat aatcgtctaa ccatataaat gtttttaatt tgaagaaatt caatattatc      600
atttacaatt tagatcattt ggacagttaa ttcctaacat gaaaggggtt aaaaaatata      660
ttttattcca aaaaacacaa cacaaatgta gacactcata acgcgtatac tcaacgcaca      720
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cgtcacactg tcaataggta tatagtctac cactgaaaaa tttaatagcc ataaatacga      840
ttacatatat caagtctata acttgaacct agatgggtta gttccaccat atataacgaa      900
tctaaccaat tgagctactc tatcatttgg aagtttttaa tttgaagaaa tttaaaatgt      960
tctttcggaa atagaatttt aaatcgagcc tatctctcgt tgactcagat cactccaatc     1020
atcatgtccc taagccggga agcagcatta accaataaaa tcttaagcaa gtgcatctag     1080
ctcacacgta ctagtacatt aattctctag ggttatatat atttgactg cagctagttg     1140
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<210> 47

<211> 1221

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0220 - RFL

<400> 47

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tcaaattttt atttttttat acaatcatta tgccttatta tatgtttatc ottcttagac	180
taatgtttac atagagttta aatcaaccac agaatcaaca agattaacct tgcagtgcga	240
ttttgacatt attcgcacgc atcaaacaag ctctgtgcaca gaaacgagcg ttttagaagt	300
tcatgtacta gattgcacca acctgacaag ttctgtgtacc gctgatgcaa tttactcttt	360
tgtttatctc aaaatacaca gtatctaaaa gtattaagtt ttatattaaa aaaatacggg	420
gtctctcgat acattaatta tccagaatgg taaaataatt cttaaaaagt atattttcgc	480
cctcggaatc aaaaacattt ttgagttgcc ctacgctggg gaaaatctgc agaatacgc	540
gctaattctc tgccccacc gctcacacga ttacatggac aaccgcccac tacgataatt	600
aagggcacia ggctacggcc tcacccgtgt tcacccgtgta cgcaacaaca gtacgcatgc	660
aggagtatct tttttcttcg gaagaggaac agtgacgccg ttgctacctc caaggagaga	720
cgggacgaaa agatcgaagg acacttgaga cgtgcgccac attacaaatg cacgtacgcc	780
gtgacaggac aacacaatgt gtgggtgtat tactcctata aggcttactg tgcctacttt	840
ttatgtaccg gcacgtaata gggcttacta gaatctagac tatcaaactc tgtgtgctct	900
agcgacgcat gcagctttct acaggacagg tcgttttcac gtagtatcga tcggtagctc	960
gctcccttcc tccaagtatt ggacaaaaac caaaagctag cgtgaatgca gatacatcga	1020
tccggaccaa tgcaacgagc tatggagatt tgtcaagtca accgcgtgca gcaactatag	1080
cccaacacta cagtggccgc tatataacga gcccaacgcg acgcacagag gtgtgtgggc	1140
gaaacacaag aaacaaccgg agaaaccgag cccggccgac cgcaaggaca gcaagctagt	1200
agccatcctc gcacccagct t	1221

<210> 48

<211> 1038

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0009 - putative cellulose synthase

<400> 48

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gccatcgagt ggtgtgccga taccggcgcc tgttctttac agcctcagct agtgttggtg      60
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atacaaagat gttgaggagt ttgtacgcca cttaattttg ctcgtaacat acattgacaa     180
tcaagaggag ccatggcatt gcgatctgct tacacggcat attcttactg gatgggtgtac     240
actacttacc ctttttaatg caagcatcaa tccattgctt ttctcactgc acacctgatt     300
cgtactgaaa acgtgaaaca taaaaaaaaa aaaaaaatct agctgatgtt ggctctcggg     360
gcctcgagtc tagtttgtcc tagatggcta acctgatatg tgttggtcac gtcacgttt     420
gaaccgagaa agagtgtgtg tgtgtgtgtg tggcggtgct gctacaccag agcctccctg     480
aatcgcaatg cgtgttaacg ccagcatcgc aggatttcat ctcaactgac aggttcagat     540
ggccttcctc ctaccgtctg ccatttatac acgcagtgac ttaacgctta cagagccgg      600
atggcccggg tctccccctc gcaccatctc accagaaaaa cggtgaggcg tcaccgcaac     660
ccaccaccca aacacatcca cgtcccttca cggttggcct tcgattttgc ttcagctgca     720
ctacgacccc tccaacacat ttccctcgcg tctcggttgcg atctcacctt acgacgatct     780
cgttccagca gcagcagcat cggcagcggc ggcttgcttc cgaagcgagc aatgcatggc     840
gcgcgcggcc gcgtgcgtgc gtgccttggc ttgcgctcta atcaaaccgg gacgccccaa     900
ctcacggttg gtgcgggacg ccaccccgcc accttacgcg ccccgctcc ctgcatctga     960
tcatcaacca gctgctatat cacctagcta gccgcgcgct cctcctcgcc caccaacgtc    1020
gcttccccgg cacctcac                                     1038

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<210> 49

<211> 838

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0075 - prolamine 10 kDa

071-prom-prov.ST25.txt

```

<400> 49
cagtgatcgc tgcactggat aattataata tcagttaaaa ttgaaaataa tgcaacttca      60
tacttgcacg gtgtcagtag tgcctgccta agaaatgtgt cttgtcataa tatgattaca      120
tgaaatatgt ttacttcctc gtttctcttt atttgtaaga taaagaacta gatatgtgga      180
aagtaggata gcaaagagta tggccaaact ctaatctttg ctttattttt tgggatggac      240
ccaaaatttg tttctccttt acttctttcc ctttacaaca atgttcttta cttccaattc      300
ttattaacaa aactccaaat acatgccaaa ctgcatatgt atgtatgcta ttaaggcaca      360
tttacaaagc tccaagttta cctactcaat cattcacata tggcgatgac tcaaactctt      420
aattgttatc tggtaagctg tgacttgtgt aacacattct acaagtcca tacgaattct      480
gttcacaaaa gtttctttgt ccagctcata atttacaaaa ctgcaaaatg ccaaagcaat      540
ctggcacaac cttatcatca tattttcttt ccacgcatta aagcactggc agaattatct      600
ttgtgtagat attccaaaag tattggttga ataaatgtcc aaataaattc catgcctcat      660
gatttccagc ttatgtggcc tccactaggt ggttttgcaa aggccaaact ctttcttggc      720
ttacacagct accagcatgt ataaataggc ccctaggcaa ccattattcc atcatcctca      780
acaatattgt ctacaccatc tggaatcttg tttaacacta gtattgtaga atcagcaa      838

```

<210> 50

<211> 978

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0076 - allergen RA2

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<400> 50
aattaatgaa tgcgagagag actatgtcgt gattgtacaa ataaaaagaa cacaagaagt      60
atagtatatt ttgcaaaaac taaggtcatt catatggtca aaccaacaat tccaaataaa      120
tatagatttt gaaggacatt aaactcacac cccatgctaa caaccctcta gctgtttctt      180
tttcttttgt cacgaaataa aaaacctctt cttccacct cacataatac ctatatTTta      240
acctcacgta ttttttactt tattacaagt cgacaaagtt atagcacaaa atattcctaa      300
catttaattt gtatcattaa tttttaaaca aagtgattat gcgtgcgtac ttctcctggc      360

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071-prom-prov.ST25.txt

catatataat gccgtgcctt gttaatatataa ccctgttatc caacgtgcgc gtaaagctat	420
ctatatatat gatatttgtg tttcagaaaa ctccaatact tatatgcttc atcaggcacg	480
ttaattttctt aatttttctga aatctagtga tgcagctttc gttactgcag cttccagagc	540
attctttttta aagtatttgt tcagaactat tcttttttatt gttccacaat ttggatcact	600
atacaagcca tggaaataaa gatgaactga ttttggtacc ttgcatgcaa aactatatat	660
gtctgttgac attatgtgca tttcttttaat tttcttccac ggaaggttca aattaataaa	720
gagtgtgggtg catatcaatc cacttttacc aacttttagtc catatgatgc ccacattagg	780
cgtggatcac aattaatatt ttatatgtct acaaaaccac aagttaatct attagctcat	840
gaacagctaa aacttttatg tcccttctta aatcacttta gtctttatag ctatatataa	900
aaccaccaat agatgaagac cttaaacctt tccctctcat acaaattcag actaaagatt	960
ttctgcaaac aaaaagcc	978

<210> 51

<211> 1264

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0110 - RCc3

<400> 51

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tttcagcacc aagagcttcc ggtggctagt ctgatagcca aaattaagga ggatgccaaa	180
acatgggtct tggcgggcgc gaaacacctt gatagggtggc ttacctttta acatgttcgg	240
gccaaaggcc ttgagacggt aaagtcttct atttgcgctt gcgcatgtac aattttattc	300
ctctattcaa tgaaattggg ggctcactgg ttcattaaaa aaaaaagaat ctagcctggt	360
cgggaagaag aggattttgt tcgtgagaga gagagagaga gagagagaga gagagagaga	420
gaaggaggag gaggattttc aggcttcgca ttgcccaacc tctgcttctg ttggcccaag	480
aagaatccca ggccgccatg ggctggcagt ttaccacgga cctacctagc ctaccttagc	540
tatctaagcg ggccgacctt gtagccacgt gcctagtgtg gattaaagtt gccgggccag	600
caggaagcca cgctgcaatg gcatcttccc ctgtccttcg cgtacgtgaa aacaaaccca	660

071-prom-prov.ST25.txt

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ggtaagctta gaatcttctt gcccggttga ctgggacacc caccaatccc accatgcccc 720
gatattcctc cgggtctcggg tcatgtgatg tcctctcttg tgtgatcacg gagcaagcat 780
tcttaaacgg caaaagaaaa tcaccaactt gctcacgcag tcacgctgca ccgcgcgaag 840
cgacgcccga taggccaaga tcgcgagata aaataacaac caatgatcat aaggaaacaa 900
gcccgcgatg tgcgtgtgac agcaatcttg gtcatttgcg ggatcgagtg cttcacagct 960
aaccaaatat tcggcgcgat atttaacaca ttatcagcgt agatgtacgt acgatttggt 1020
aattaatcta cgagccttgc tagggcaggt gttctgccag ccaatccaga tcgccctcgt 1080
atgcacgctc acatgatggc agggcagggg tcacatgagc tctaacggtc gattaattaa 1140
tcccggggct cgactataaa tacctcccta atcccatgat caaaaccatc tcaagcagcc 1200
taatcatctc cagctgatca agagctctta attagctagc tagtgattag ctgcgcttgt 1260
gatc 1264

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<210> 52

<211> 1808

<212> DNA

<213> *Oryza sativa*

<220>

<221> misc_feature

<223> PRO0133 - chitinase Cht-3

<400> 52

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tttggcgcgg ggcagaagag tggactttaa ctttcttttt aataaaatct ccaattaata 60
tgtaattata atatactttt aatcaaaaca tgcaaagcta gcagtattta catcactaga 120
agtaaattctt tcttgctcat gatgcttcag ccggacggaa ccctaaaata tagatggggc 180
ggatacactc gattaaaaca gctaattgca acacatatca tataagggtt tggaattcoat 240
accaaagctc ccgaaattcg tctatttcga tgaggcccaa gacatgacct cctgtttcgc 300
ccatagttta tgggtgtttg taaaatttgg ttaaaatctg tctattttag taggtcccca 360
aattcttatg caattgaatc ctagaacct atcatattta tattgcaatt gcacaaaaat 420
aatgtgcaat caatatattc caattgcaat acatatcaag catgagggtg aatacatatc 480
cagccgctag cactgggtct gttgaggtgc ttcttgacg aacagctgca atctgtttgg 540
ctaggctgtt ggcgccaggc actgctgtcg tgctgcaaca atggcacatt cgtcgagcac 600
acaaccgcgc ctatgcacag cgcaagctcg ctgccttggc ccgtggttcc agtgttgcat 660

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071-prom-prov.ST25.txt

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caagaagcag aagaggagtc gggtcgggag tgtggggcga cgtaggctgt agctgggccg	900
gccgccccag gccgcctaata gagggtgtcc gccctggcc tgacacgatg ggtaattaaa	960
tagttatgca tgcctctctt tgtctaaaca atatgtataa aattgacgat atcttgggca	1020
aatcactgg gcatggcaca caggagagct acttttagcga catgaatcta ggcgaaaatc	1080
tattgaacca aaaatcgact gtaatctcat gaaaattttc gtcataatta tagcaaaatc	1140
gttggtggat tgattgcacg agaaaacaga agaaggagc taggtgatat tatattgttt	1200
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gagatcaatc tatcaatcgt caaccctttg cctttgttag cgagctagcg tgtacacatt	1440
tcaattatat atgggtgcag catggcatcc acgcctccac ggtcaacgtg gaaatatctc	1500
tggaaactta ctttttctaa ataactgaac ggattggagg caggagacaa atttgaccaa	1560
cacaatatat ccacgacggc tagacaatac tagtagatgc atgcatggaa ggatatagta	1620
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cgatgcacca tgcatatcca tctctatata aagccatgcg atcccacga ttcttgacaa	1740
cacactagct acttctactt ctatcatacc aaacaaacta gcttaatttg cattgcatca	1800
cattgccg	1808

<210> 53

<211> 1139

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0141 - cyclophilin 2

<400> 53

tgacaaaata atactctatc catcctaaaa tataaggaat tttatgtgga tgtgacgtat	60
cttatacttt ggggtgagagg aagagggtact aataccacta ttatattgta ctccctccat	120

071-prom-prov.ST25.txt

actcgtaaaa gaaatcgttt aggacagoga cacggtttcc aaaacacaaac tttgacttct	180
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caagacaaat ctatatatat aattttttata ttttcaaact caacaactta aaagttattt	300
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<210> 54

<211> 1052

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PR00091 - prolamine RP5

<400> 54

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caatctccta ttctcggcac gtgtgatata caatggtaag tgagatatac aattctcggc	180
acggctacat tacaaggtgt cgcattgtgt caatgtttgg ttaatttgct agattcacat	240
aatacatgcc aggaagttca gaacaatgtg ttgcctttca ccggaaaact ttgttgagc	300

071-prom-prov.ST25.txt

aaatgccttc ttcttttttg cttctgcttc ttgagtcctat gtggaggaag cagtagatag	360
ctgatgatat caggattcct tctgtgtctg tgtagggtga gcaacaccac tataattttt	420
atthagcaac acaatatcaa ttgggtctat aaaagtatga attaaatcaa tccccaacca	480
caattagagt aagttggtga gttattgtaa agctctgcaa agttaattta aaagttattg	540
cattaactta tttcgtatca caaacaagtt ttcacaagag tattaatgga acaatgaaaa	600
ccattgaaca tactataatt ttttttttta ctgaaattat ataattcaaa gagcataaac	660
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acttagatcc taatatagca tcaagcaaaa ctaacactct aaagcaaccg atagggaaac	960
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<210> 55

<211> 883

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0001 - metallothionein Mte

<400> 55

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attaatctat atttaatact taatactttt gtccgtgtat gtaaaaaaat tttgacccaa	480
caactgaaca cggcctgtat atgtaaccaa agaaagatca aaggagagga ggtagctact	540

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cctacaaaga aagaagaaac gatgagattt gattgcctac tactccagct agctagtata	600
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<210> 56

<211> 688

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0077 - prolamine RP7

<400> 56

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aaaagcgcct ggcgtagcat aattttgtta ttcttggtga aatttgtaat ttaagatgca	120
tgagaccaca cgaccttaat gttcaacgtg tcatgcatta gtgaaataat agctcacaaa	180
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ctaactctct aaagcaaccg atgaggaatt gaaagaaaat acgtgccacc ggatctataa	600
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aaatagaaag atctagtgtc ccgcagca	688

<210> 57

<211> 1258

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0080 - metallothioneine-like ML2

<400> 57

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aatcatttttt tttgaaagtt taatcaagtt tataaaaaaa aatataacaa cacttatagc	120
cttaaattag tttcattacg tcttacattg aacatatttt gatattttct gttaaaaata	180
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cacaatagtt ggttttctgt gcatatgtga ttttaactgtg tattcttctc gagttgctag	420
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<210> 58

<211> 668

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0090 - prolamine RP6

<400> 58

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<210> 59

<211> 1245

<212> DNA

<213> Oryza sativa

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<223> PRO0126 - metallothionein RiCMT

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<210> 60

<211> 2195

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0129 - GOS2

<400> 60

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<211> 1008

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0135 - alpha-globulin

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<210> 62

<211> 1395

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0136 - alanine aminotransferase

<400> 62

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1395

<210> 63

<211> 2115

<212> DNA

<213> Hordeum vulgaris

<220>

<221> misc_feature

<223> PRO0146 - sucrose synthase SS1 (barley)

<400> 63

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<211> 342

<212> DNA

<213> Hordeum vulgare

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<221> misc_feature

<223> PRO0147 - trypsin inhibitor ITR (barley)

<400> 64

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<210> 65

<211> 2753

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0149 - ubiquitin 2 with intron

<400> 65

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<210> 66

<211> 1828

<212> DNA

<213> Oryza sativa

<220>

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<223> PRO0151 - WSI18

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<210> 67

<211> 1553

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PRO0175 - RAB21

<400> 67

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<210> 68

<211> 1236

<212> DNA

<213> *Oryza sativa*

<220>

<221> misc_feature

<223> PRO0218 - oleosin 18 kDa

<400> 68

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<210> 69

<211> 1488

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> PR00219 - ubiquitin 2 without intron

<400> 69

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1488

<210> 70

<211> 55

<212> DNA

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<223> forward primer PRM2768

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ggggacaagt ttgtacaaaa aagcaggctc ccgatttagt agaccacatt ttggc

55

<210> 71

<211> 57

<212> DNA

<213> Artificial sequence

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<223> reverse primer PRM2769

<400> 71

ggggaccact ttgtacaaga aagctgggctc gtgtagaaaa tcttaacccg aaaatcg

57

<210> 72

<211> 54

<212> DNA

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<223> forward primer PRM2422

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54

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<211> 58

<212> DNA

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<223> reverse primer PRM2425

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56

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54

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53

<210> 87

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<223> reverse primer PRM2427

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<223> forward primer PRM2855

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<223> forward primer PRM2857

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<211> 55

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<223> forward primer PRM3023

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<223> reverse primer PRM3024

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<223> forward primer PRM3025

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<210> 97

<211> 49

<212> DNA

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<223> reverse primer PRM3026

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<210> 98

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<211> 58

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<210> 100

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<223> forward primer PRM3779

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<223> reverse primer PRM2166

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<211> 55

<212> DNA

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<223> forward primer PRM4503

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<223> reverse primer PRM4504

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<223> forward primer PRM2937

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<210> 109

<211> 56

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<223> reverse primer PRM2938

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<211> 49

<212> DNA

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<220>

<223> forward primer PRM3270

<400> 110

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<210> 111

<211> 57

<212> DNA

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<223> reverse primer PRM2940

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<210> 112

<211> 55

<212> DNA

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<220>

<223> forward primer PRM2939

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<210> 113

<211> 57

<212> DNA

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<220>

<223> reverse primer PRM2940

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<210> 114

<211> 57

<212> DNA

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<220>

<223> forward primer PRM3031

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<210> 115

<211> 54

<212> DNA

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<220>

<223> reverse primer PRM3032

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<210> 116

<211> 53

<212> DNA

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<223> forward primer PRM3051

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<210> 117

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<212> DNA

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<220>

<223> reverse primer PRM3052

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<212> DNA

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<223> forward primer PRM3592

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<210> 119

<211> 60

<212> DNA

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<212> DNA

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<223> forward primer PRM5131

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<212> DNA

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<223> reverse primer PRM2195

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<223> forward primer PRM3782

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<210> 123

<211> 58

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<210> 124

<211> 54

<212> DNA

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<220>

<223> forward primer PRM2840

<400> 124
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<210> 125

<211> 54

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<211> 3410

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<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC84853 + TC90672

<400> 210

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<211> 1569

<212> DNA

<213> Oryza sativa

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<223> TC clone TC52979

<220>

<221> misc_feature

<222> (1005)..(1005)

<223> unknown nucleotide

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<213> *Oryza sativa*

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<210> 213

<211> 799

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC45098

<400> 213

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<210> 214

<211> 662

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC39230

<400> 214

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<211> 3173

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC83117

<220>

<221> misc_feature

<222> (2792)..(2792)

<223> unknown nucleotide

<400> 215

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<211> 1488

<212> DNA

<213> *Oryza sativa*

<220>

<221> misc_feature

<223> TC clone TC89913

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<211> 1170

<212> DNA

<213> Oryza sativa

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<222> (1162)..(1162)

<223> unknown nucleotide

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<211> 861
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<211> 2957

<212> DNA

<213> Oryza sativa

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<223> TC clone TC52500

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<210> 222

<211> 686

<212> DNA

<213> Oryza sativa

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<211> 826

<212> DNA

<213> Oryza sativa

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<211> 2739

<212> DNA

<213> Oryza sativa

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atgatgtggc cttaaacctg agctgcacaa gcctaattga aaaataaagt ttcaggcttt	2700
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<210> 226

<211> 612

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC63273

<400> 226

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accatgtcgg acaagtgcgg caactgcgac tgtgctgaca agagccagtg cgtgaagaaa	180
ggtagcagct atggcgctcgt catagttgaa gccgagaaga gccacttcga ggaggtcgcc	240

071-prom-prov.ST25.txt

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ttagtccaca ataaaaacca cccatatgag tgtgggttgt gtgtgtgctg attattgctg 420
aattaaaccc gtgtgttatc gattaattaa gcagctggtc gcttcggcga ctatgcgtgt 480
atcaacgtat gaatttgtgt gatgtgatgt acccgtgcta ttctcagtga aattaatcaa 540
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atttcttggt ct 612
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<210> 227

<211> 1252

<212> DNA

<213> Oryza sativa

<220>

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<220>

<221> misc_feature

<222> (5)..(5)

<223> unknown nucleotide

<400> 227

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cgcccacagc gacatcgaca gcaccaacaa gacgctgctc aagagcgacg ccctgtacaa 180
gtatgtcctg gacacgacgg tgctgccacg ggagccggag tgcattgcgcg atctgcgcct 240
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gggatgtgtc tgaagatggc cggagcgaag aggacaatcg aggtgggtgt cttcaccggc 360
tactcgtgtc tggcgcgagg gctggcgctg ccggaggacg ggaaggtggg ggcgatcgac 420
ccggacaggg agagctacga gatcgggcgg ccgttcttgg agaaggccgg ggtggcgcac 480
aaggtggact tccgcgaggg gaaggggctg gagaagctgg acgagctgct cgccgaggag 540
gcggcggcgg ggcgcgaggg ggcgttcgac ttcgcgttcg tggacgcgga caagcccaac 600
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071-prom-prov.ST25.txt

tacgtcaagt accacgagca gctgctgcag ctggtgcgcg tcggcgggca catcgtgtac	660
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gaccggaggt tctccgtcgc catcagggac ctcaactcca ggctcgccgc cgacccgcgc	780
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<210> 228

<211> 750

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC56720

<400> 228

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tgttactcaa gtttacaggc aatatcagct gcagccgat ctcatgctgc agcaacagat	180
gcttagccca tgcggtgagt tcgtaaggca gcagtgcgc acagtggcaa ccccttctt	240
ccaatcaccg gtgtttcaac tgagaaactg ccaagtcatt cagcagcagt gctgccaca	300
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gcagcagcta cagctacaac agtttgctgg cgtctacttc gatcaggctc aagctcaagc	420
ccaagctatg ttggccctaa acttgccgtc aatatgcggt atctacccaa gctacaacac	480
tgctccctgt agcattccta ccgtcgggtg tatctgggtat tgaattgtag cagtatagta	540
gtacaggaga gaaaaataaa gtcatgcatc atcgtgtgtg acaagttgaa acatcggggg	600
gatacaaatc tgaataaaaa tgcatgcaa atttaaactc aatgcctctg taaggataaa	660

tcctagtaca ttgttgaaat taattatcat caccgtctac tatggttgca tttacacttt 720
 acgatgtgtt cctacatttg gttctaagct 750

<210> 229

<211> 743

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC56718

<400> 229
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 gcggtttgat cctcttagtc aaagttatag gcaatatcaa ctacagtcgc atctcctact 180
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 aaccccttc tggcaaccag ctacgtttca attgataaac aaccaagtca tgcagcagca 300
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 aacaatataa tagttcgat gttaaaaata agtcataca tcatcatgtg tgactgttga 600
 aacttagggg catataaatc taaataaaat catcttacct atttaaatag cattcctcta 660
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<210> 230

<211> 671

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC90434

<220>

<221> misc_feature

<222> (3) .. (3)

<223> unknown nucleotide

<220>

<221> misc_feature

<222> (14) .. (14)

<223> unknown nucleotide

<400> 230

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tttgatgctc ttagtcaaag ttatagacaa tatcaactac aatcgcatct cctgctacag	180
caacaagtgc tcagcccatg cagtgaagttc gtaaggcaac agcatagcat agtggcaacc	240
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tgccaacagc tcaggctggg agcgcaacaa tctcactacc aggccattag tagcgttcag	360
gcgattgtgc agcaactaca gctgcagcag gtcgggtgttg tctactttga tcagactcaa	420
gctcaagctc aagctttgct ggccttaaac ttgccatcca tatgtggtat ctatcctaac	480
tactacattg ctccgaggag cattcccacc gttgggtggg tctgggtactg aattgtaata	540
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<210> 231

<211> 709

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC83072

<400> 231

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cgcgccggcg cgagctcccg gcgttctgca agtggacatc cccaacggcg gaggtggtgt	540
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gtggtgtggt actaaaataa tagtgttttc cttttgtttg gaagcatatg tgtggtgaat	660
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<210> 232

<211> 436

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC90038

<400> 232

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436

<210> 233

<211> 3180

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC56736

<400> 233

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<210> 234

<211> 1254

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC89839

<400> 234

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<210> 235

<211> 463

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC61377

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 gcatggcggt gctggcgctg cttctgctgc tcggccacca cgcggcggcc atcgaggcca 180
 acgtcggcgt caccgccag gccagcttca gcctgaacgg cggcgccggc gccggcgcg 240
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<210> 236

<211> 549

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC60065

<400> 236
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071-prom-prov.ST25.txt

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tcagtgtgtg tgtgtgtttg tgtcaaccat gtgtgaatca ggtgtcaaaa atcgtggctg	480
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<210> 237

<211> 515

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> new sequence

<400> 237

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cccatggctg cagctgtggc tccagctgca agtgcaacc ctgcaactgc taaggccaag	300
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ccatccagtg tgatggctca tgccctgtgtg tgtgtgtgtt tgtctcaacc atgtgcgaat	420
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<210> 238

<211> 1272

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> new sequence

<220>

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<222> (17)..(17)

<223> unknown nucleotide

<220>

<221> misc_feature

<222> (50)..(50)

<223> unknown nucleotide

<400> 238

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<210> 239

<211> 860

<212> DNA

<213> Oryza sativa

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<223> TC clone TC85840

<220>

<221> misc_feature

<222> (1)..(1)

<223> unknown nucleotide

<220>

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<222> (10)..(10)

<223> unknown nucleotide

<400> 239

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<210> 240

<211> 1167

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC65908

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<210> 241

<211> 871

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC56634

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tcaaggggtgt cggcgcgcagg ttctccaaca tcgcctgcaa gaaggccgac atcgacatga	240
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acccgcggca gttcaaggtg ccgactggt tcctcaacag gaagaaggac tacaaggacg	360
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<210> 242

<211> 1245

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC89846

<400> 242

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<210> 243

<211> 1416

<212> DNA

<213> Oryza sativa

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<220>

<221> misc_feature

<223> TC clone TC82935

<400> 243

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<210> 244

<211> 545

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC82977

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<210> 245

<211> 978

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC90377

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<210> 246

<211> 362

<212> DNA

<213> Oryza sativa

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<222> (205)..(205)

<223> unknown nucleotide

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<222> (332) .. (332)

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caatctacag ctcagatgca atcagatcca ttgccttcaa ctacatcggg gtggatggac	180
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<210> 247

<211> 1149

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC56770

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<210> 248

<211> 824

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC64259

<400> 248

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<210> 249

<211> 1962

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC64429

<400> 249

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ggaatcccca gtctcttggt cagaagccag ttacattctt cagggagggt attgctcttt	300
gtgatcatcc atgcttggtg gaaaaggagg aaaccaaatc attgttcagt gctgatgcca	360
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<211> 2004

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> new sequence

<400> 250

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071-prom-prov.ST25.txt

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<210> 251

<211> 1745

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC91931

<220>

<221> misc_feature

<222> (1719)..(1719)

<223> unknown nucleotide

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<400> 251
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```


071-prom-prov.ST25.txt

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gaaggttcac tctactaca gcttcgctcc tctcactgcc tgcttggccg tcaactactt 540
ggaccgcttc ctctcactct accagcttcc ggatggcaag gattggatga cgcagctgct 600
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ccagatgtcc tgcgtgtgtt tcagttttgc gttgttctgt aatttcccct gcttaattcc 1680
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tcgag 1745

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<210> 252

<211> 1659

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC65369

<400> 252
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 ggcacgaggc ggaggccgcg gaggaggtgg tgggaatcga tccctttctg ttccggccgc 180
 ctcgatcgcc cgctaccgcg acgccgcctt ttcgtcccgg tggatttctg cctccctttt 240
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 ggcggcgact gggagttatc cattgctgac gacgaccatg tgttgctcat ggaccgggac 480
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<210> 253

<211> 912

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC90666

<400> 253

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gcgaacgccg ggccaacac taacgggtcc cagttcttca tctgcaccgt gccctgcagc      480
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gccatcgaga aggtgggatc ccgcggcggg agcaccccca agccggtcgt catcgccgac      600
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ttgagtcgtc gagtccctaa ataaggagga ggtgggtggtg gtgttagtct ttttatgagt      720
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gatcgattcg gtggagttag atctgcgata cttcttgggg ttggttttaa atcttaattc      840
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```

<210> 254

<211> 683

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC96414

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<400> 254
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attgtaaaac tagcgaaaca acgtgcagtt catcttggtt ttccagctcc agctagcgct      180
tccatggggt ccaagaagga tgccccaacc tgggcagacc agtggggctc atctgggtggc      240
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ggccagtacc agaagagggc tggaaagtga ttggatttct gatgcgtttg cctggttgct      480
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agagacggga ggttatgtct ttggcgctat tcatttggat gatcttgtga gcaattaatt      600
aaactgttgc tgattttaat acaagagagt gtcttttggg gcaacgattt ttgttcgatt      660
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```

<210> 255

<211> 478

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC83635

<220>

<221> misc_feature

<222> (399)..(399)

<223> unknown nucleotide

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<400> 255
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ggagccgcag gaatggcggc gatgcagagc tggcgcaagg cgtacggcgc gctcaaggac      120
tccaccaccg tcagcctcgc caacctcaac tccgacttca aggatctgga tgtggcgatc      180

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071-prom-prov.ST25.txt

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<210> 256

<211> 1854

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC82859

<400> 256

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 ggcaagcagc tggaggatgg caggaccctt gctgactaca acatccagaa ggagtccacc 960
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071-prom-prov.ST25.txt

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gaggatggcc gcaccctcgc cgactacaac atccagaagg agtctaccct ccacctggtg	1200
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<210> 257

<211> 981

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC89681 + TC89680

<400> 257

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071-prom-prov.ST25.txt

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cgtgctccag caggcagggg agcaggtgaa gagcgtggcg gtgggggcga aggacgcggt	660
gatgtacacg ctcgggatgt caggcgataa caagaacaac gccgctgccg gcaaggacac	720
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gaattcgacg tggtcgggtg cacacttttg tttttcagtt gtgctcaact gttcatgttt	900
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<210> 258

<211> 694

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC89946

<220>

<221> misc_feature

<222> (413) .. (413)

<223> unknown nucleotide

<400> 258	
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gctgaagagg aggcaaagag agcgtaacga tggttaagat tgtggtgaac tgnaaggcat	420
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071-prom-prov.ST25.txt

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ggttgccatg catacaaaga atgaggaata catctattcg ggtgtacagt tgtatgaaaa	660
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<210> 259

<211> 732

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC85888

<400> 259	
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ttcacgtaag atcgagcagt gatttcagtc agtatgtatg ctcaaatga tttagttgta	660
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<210> 260

<211> 1203

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC89848

<400> 260

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<211> 964

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC89670

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ccgcttttctt cgtttttatg gaggagttcc gtaaggagtt caaggagaag aacccaaga      360
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<210> 262

<211> 1542

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC82827

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<210> 263

<211> 1432

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC89636

<220>

<221> misc_feature

<222> (1429)..(1429)

<223> unknown nucleotide

<400> 263

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<210> 264

<211> 659

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC82749

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<210> 265

<211> 2306

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC89647

<220>

<221> misc_feature

<222> (7)..(7)

<223> unknown nucleotide

<220>

<221> misc_feature

<222> (96)..(96)

<223> unknown nucleotide

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<222> (2297)..(2297)

<223> unknown nucleotide

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<210> 266

<211> 1310

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC82826

<400> 266

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<210> 267

<211> 2695

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC89792 + TC89796

<220>

<221> misc_feature

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<223> unknown nucleotide

<400> 267

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<210> 268

<211> 2271

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC82833

<220>

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<222> (59) .. (59)

<223> unknown nucleotide

<400> 268

071-prom-prov.ST25.txt

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071-prom-prov.ST25.txt

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<210> 269

<211> 2599

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC90370

<220>

<221> misc_feature

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<223> unknown nucleotide

<220>

<221> misc_feature

<222> (1687)..(1687)

<223> unknown nucleotide

<400> 269

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071-prom-prov.ST25.txt

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<210> 270

<211> 1226

<212> DNA

<213> Hordeum vulgaris

<220>

<221> misc_feature

<223> TC clone TC27215

<400> 270

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071-prom-prov.ST25.txt

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<210> 271

<211> 2002

<212> DNA

<213> Hordeum vulgaris

<220>

<221> misc_feature

<223> TC clone TC27238

<400> 271
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071-prom-prov.ST25.txt

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<210> 272

<211> 1089

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC82803

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071-prom-prov.ST25.txt

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<210> 273

<211> 1515

<212> DNA

<213> *Oryza sativa*

<220>

<221> misc_feature

<223> TC clone TC84300

<400> 273
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<210> 274

<211> 939

<212> DNA

<213> Oryza sativa

<220>

<221> misc_feature

<223> TC clone TC83646

<400> 274

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071-prom-prov.ST25.txt

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